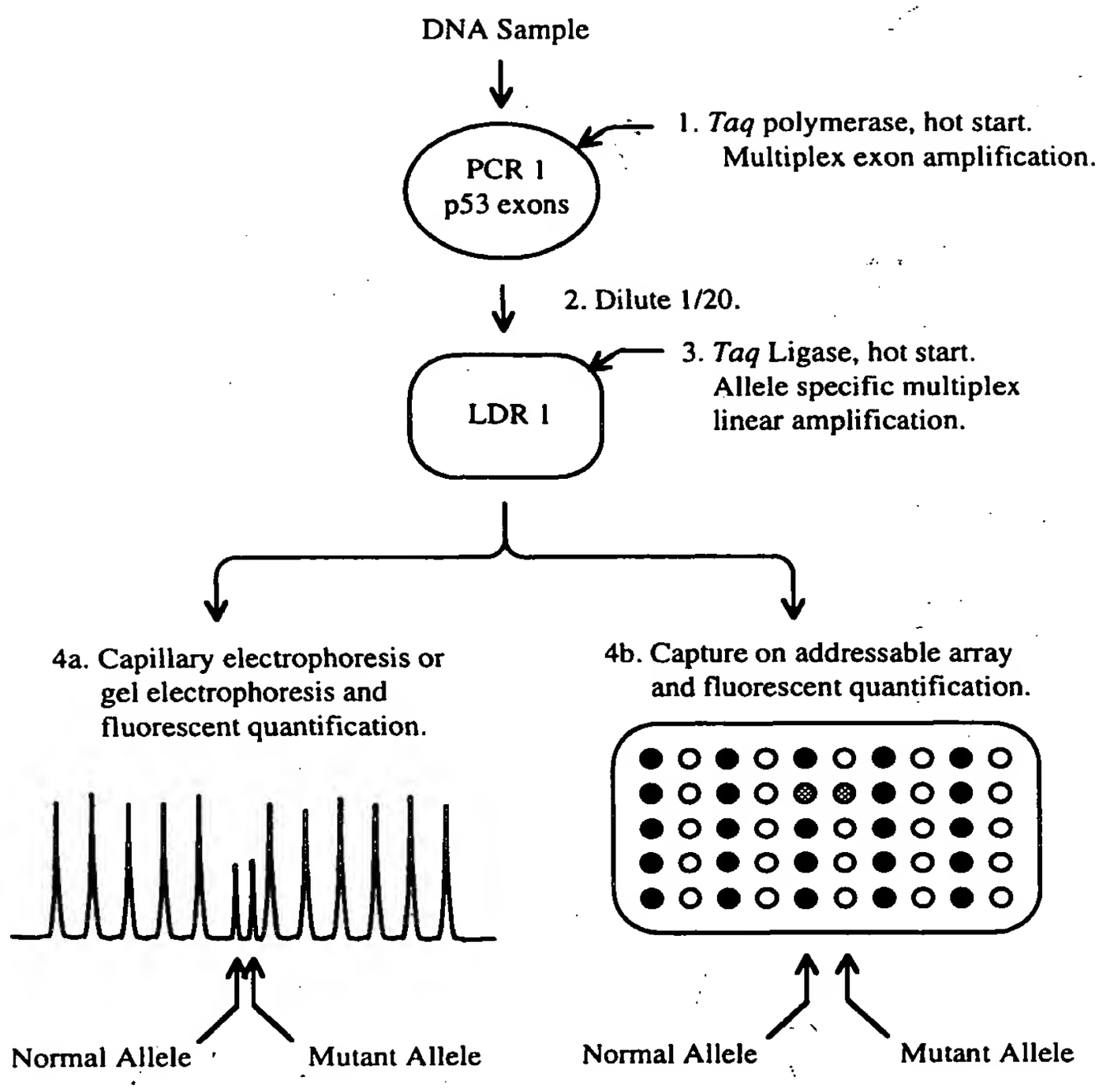

DNA Sample
PCR 1
p53 exons
1. *Taq* polymerase, hot start.
Multiplex exon amplification.
2. Dilute 1/20.
LDR 1
3. *Taq* Ligase, hot start.
Allele specific multiplex
linear amplification.
4a. Capillary electrophoresis or
gel electrophoresis and
fluorescent quantification.
4b. Capture on addressable array
and fluorescent quantification.
Normal Allele
Mutant Allele
Normal Allele
Mutant Allele

FIG. 1

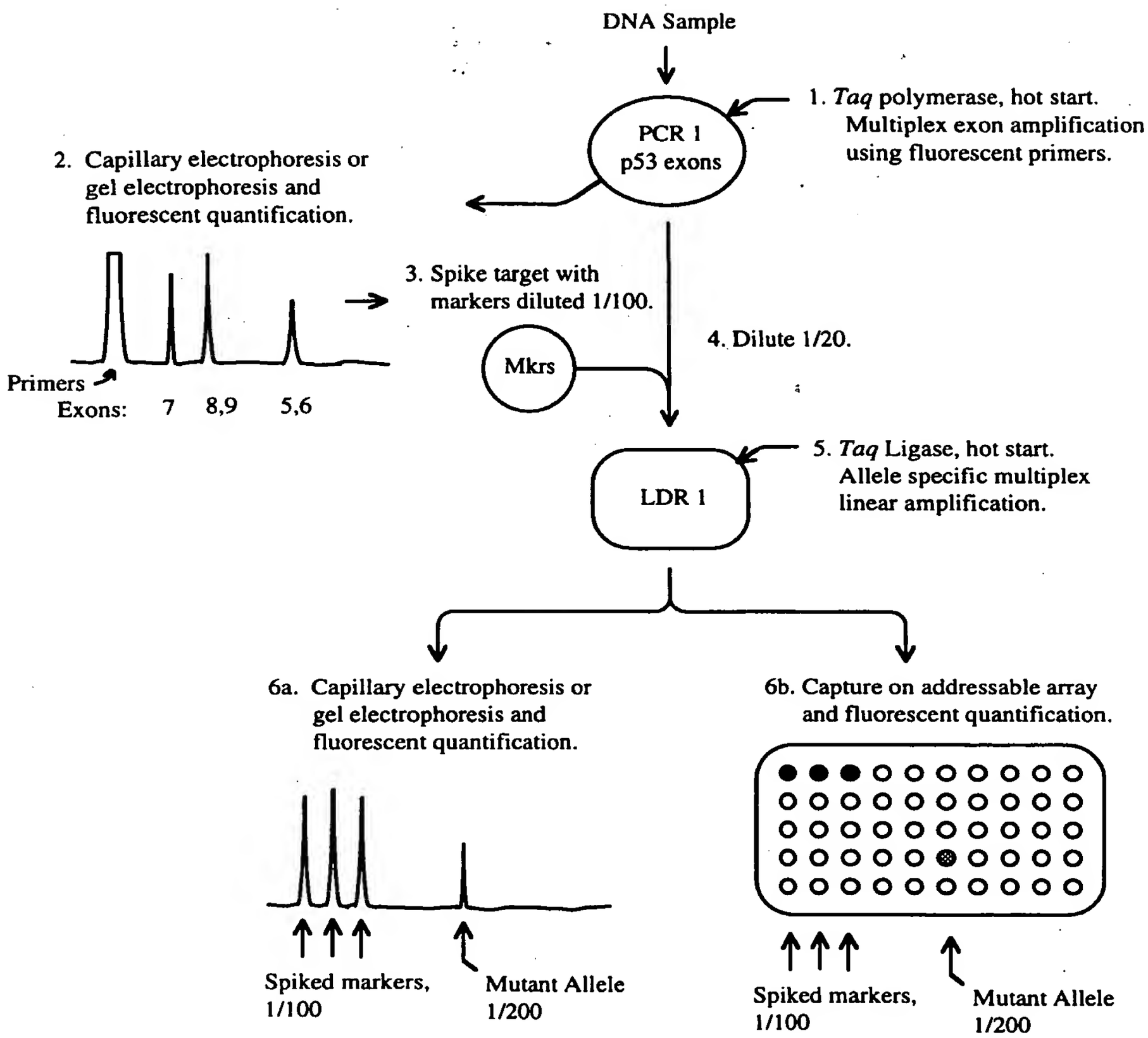
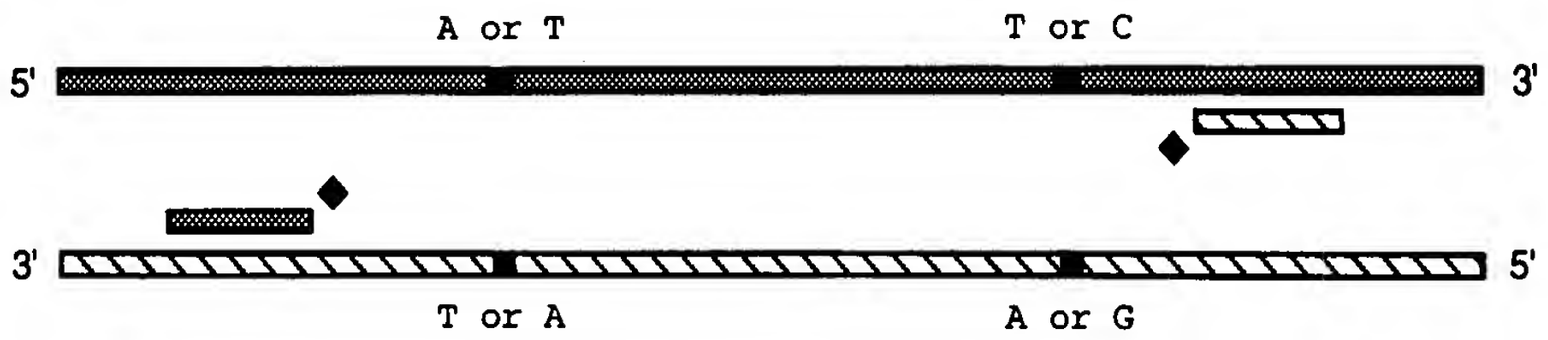

DNA Sample
PCR 1
p53 exons
1. Taq polymerase, hot start.
Multiplex exon amplification
using fluorescent primers.
2. Capillary electrophoresis or
gel electrophoresis and
fluorescent quantification.
Primers
Exons: 7 8,9 5,6
3. Spike target with
markers diluted 1/100.
Mkrs
4. Dilute 1/20.
LDR 1
5. Taq Ligase, hot start.
Allele specific multiplex
linear amplification.
6a. Capillary electrophoresis or
gel electrophoresis and
fluorescent quantification.
Spiked markers,
1/100
Mutant Allele
1/200
6b. Capture on addressable array
and fluorescent quantification.
Spiked markers,
1/100
Mutant Allele
1/200

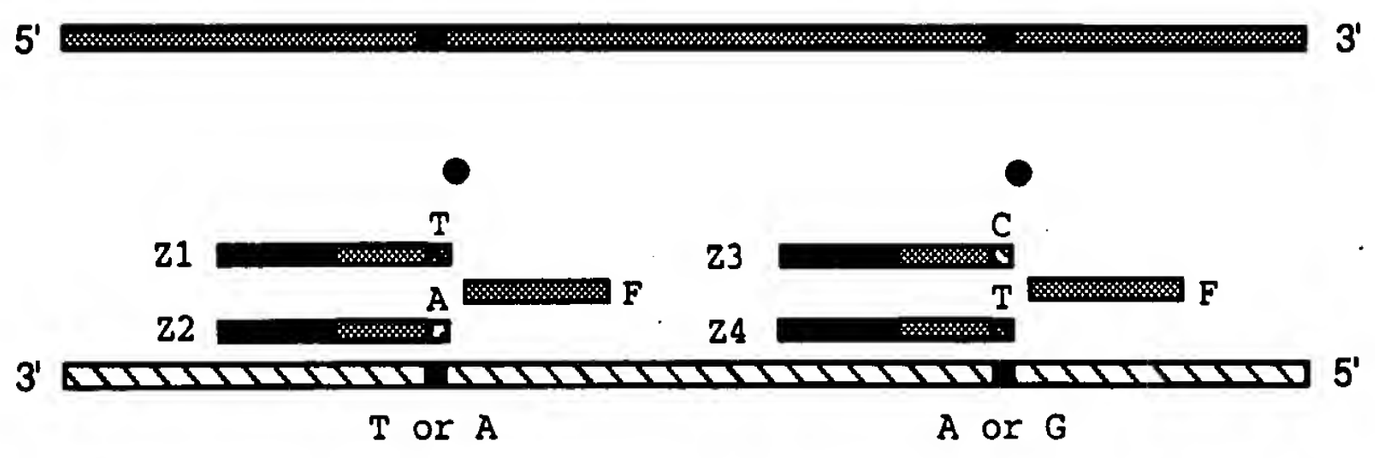
FIG. 2

PCR/ LDR

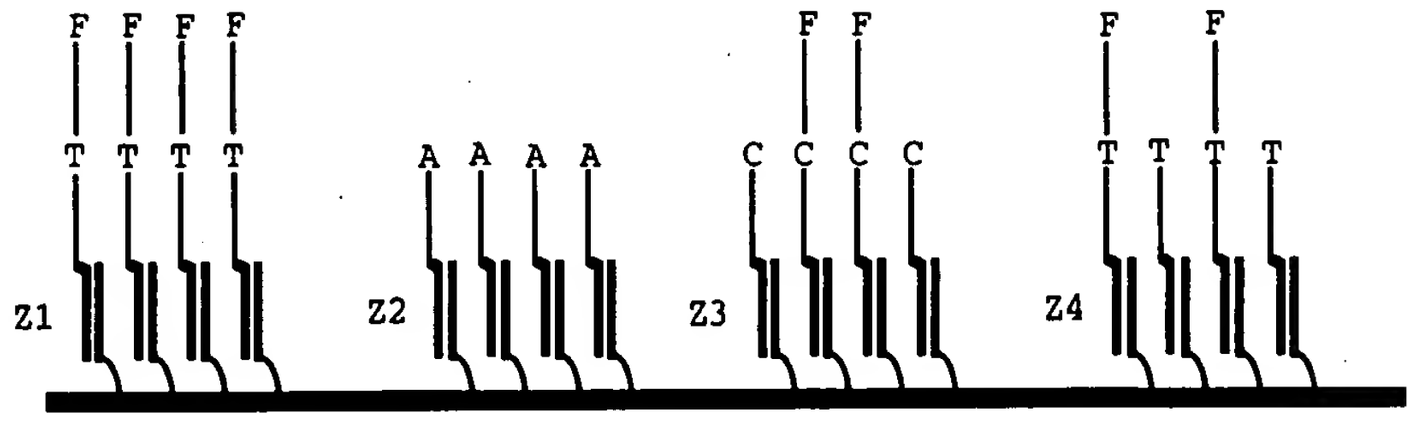
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase. ◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



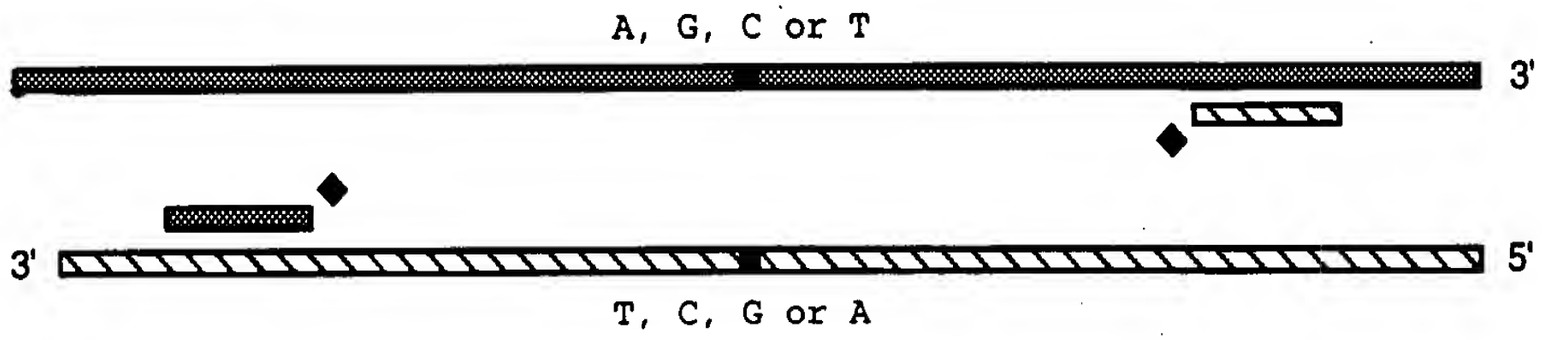
Homozygous: T allele only.

Heterozygous: C and T alleles.

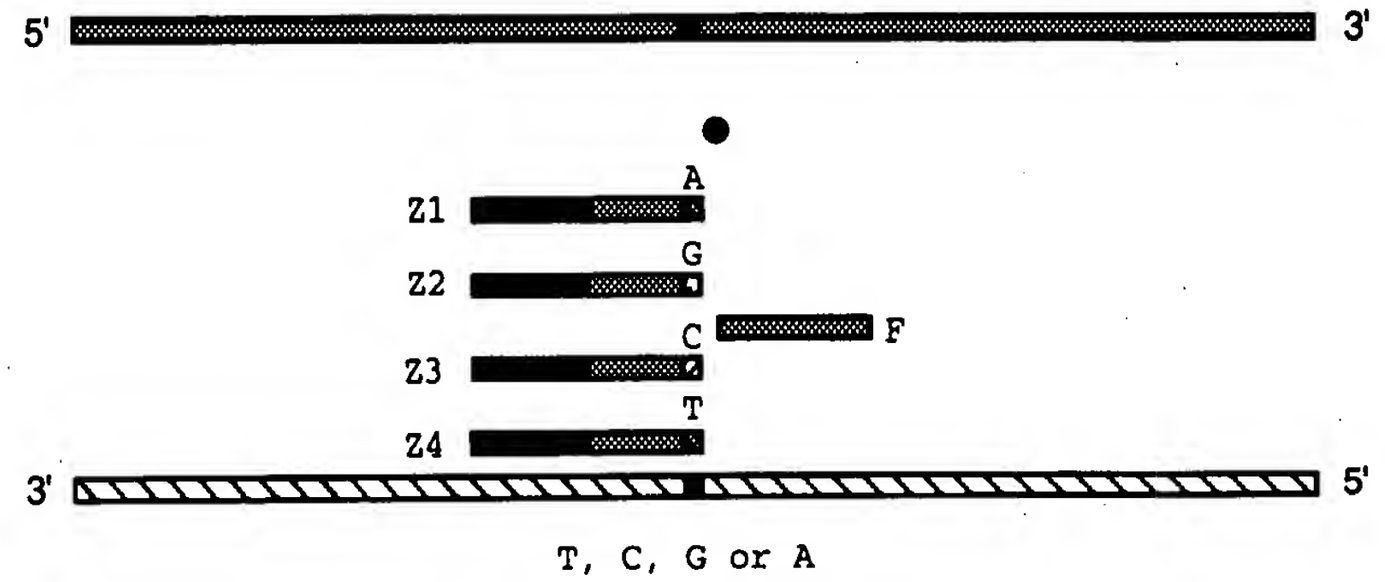
FIG. 3

PCR/ LDR

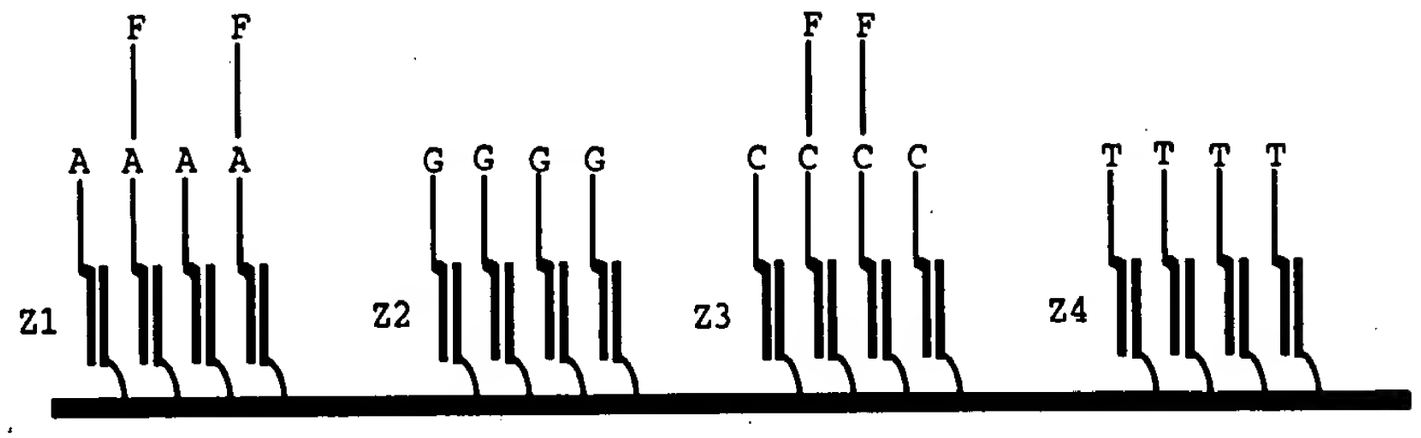
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase. ◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.

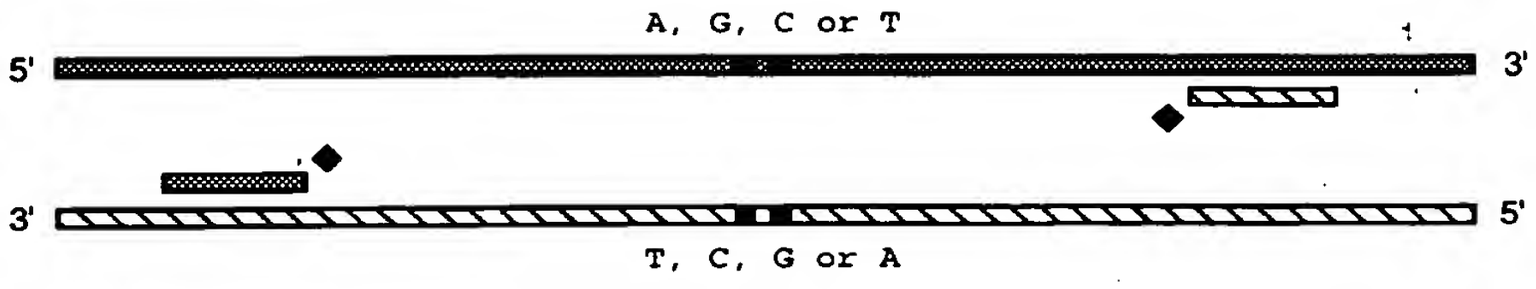


Heterozygous: A and C alleles.

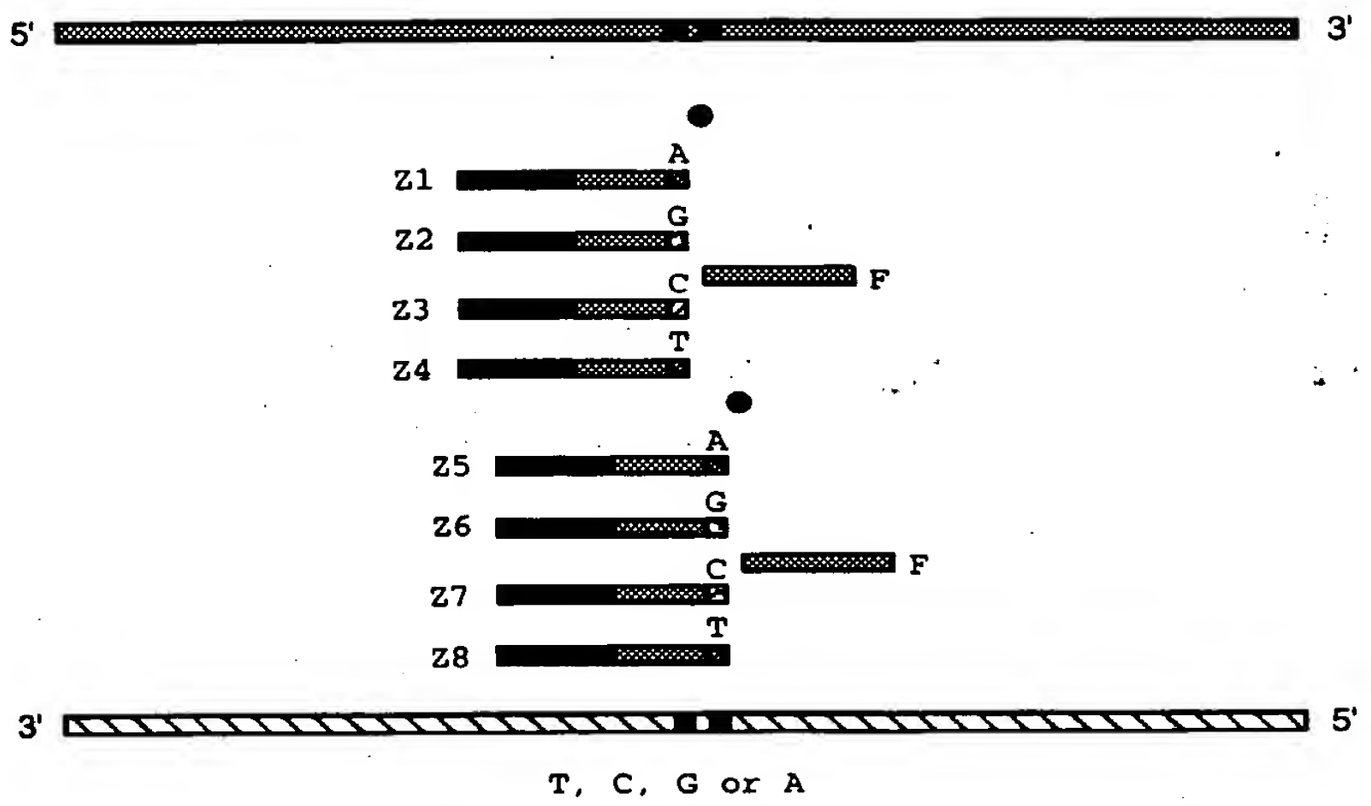
FIG. 4

PCR/ LDR : Nearby alleles

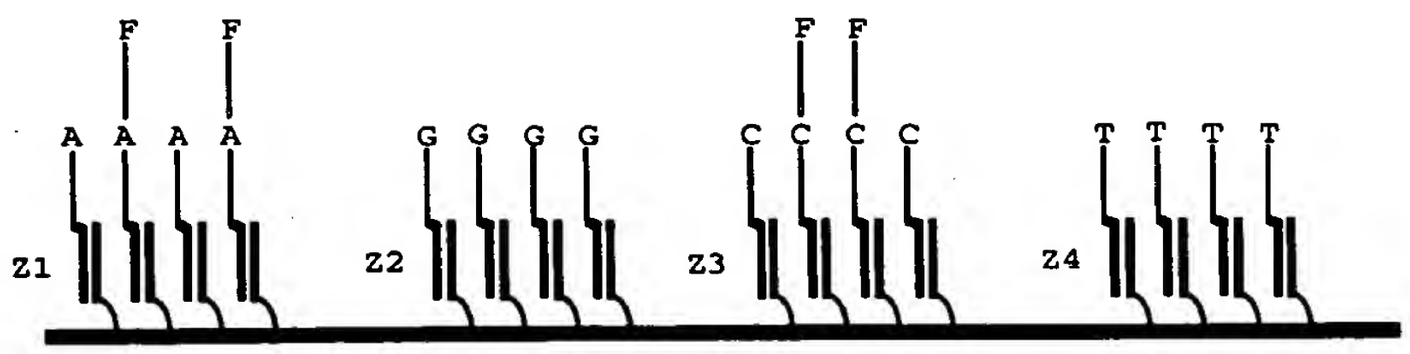
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



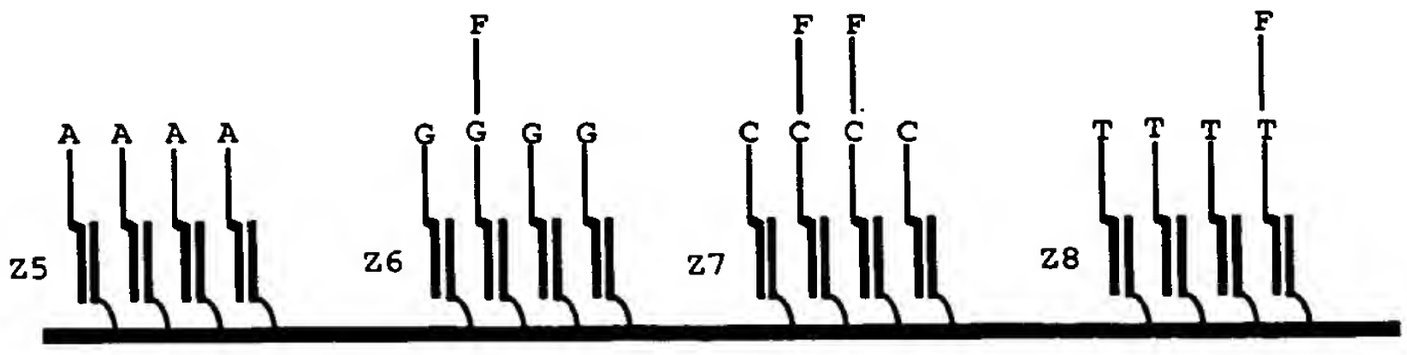
2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A and C alleles.

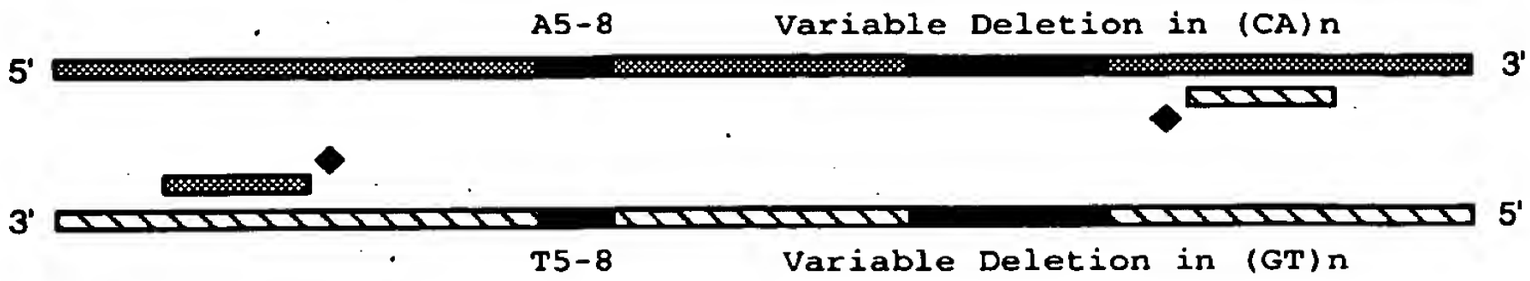


Heterozygous: G,C, and T alleles.

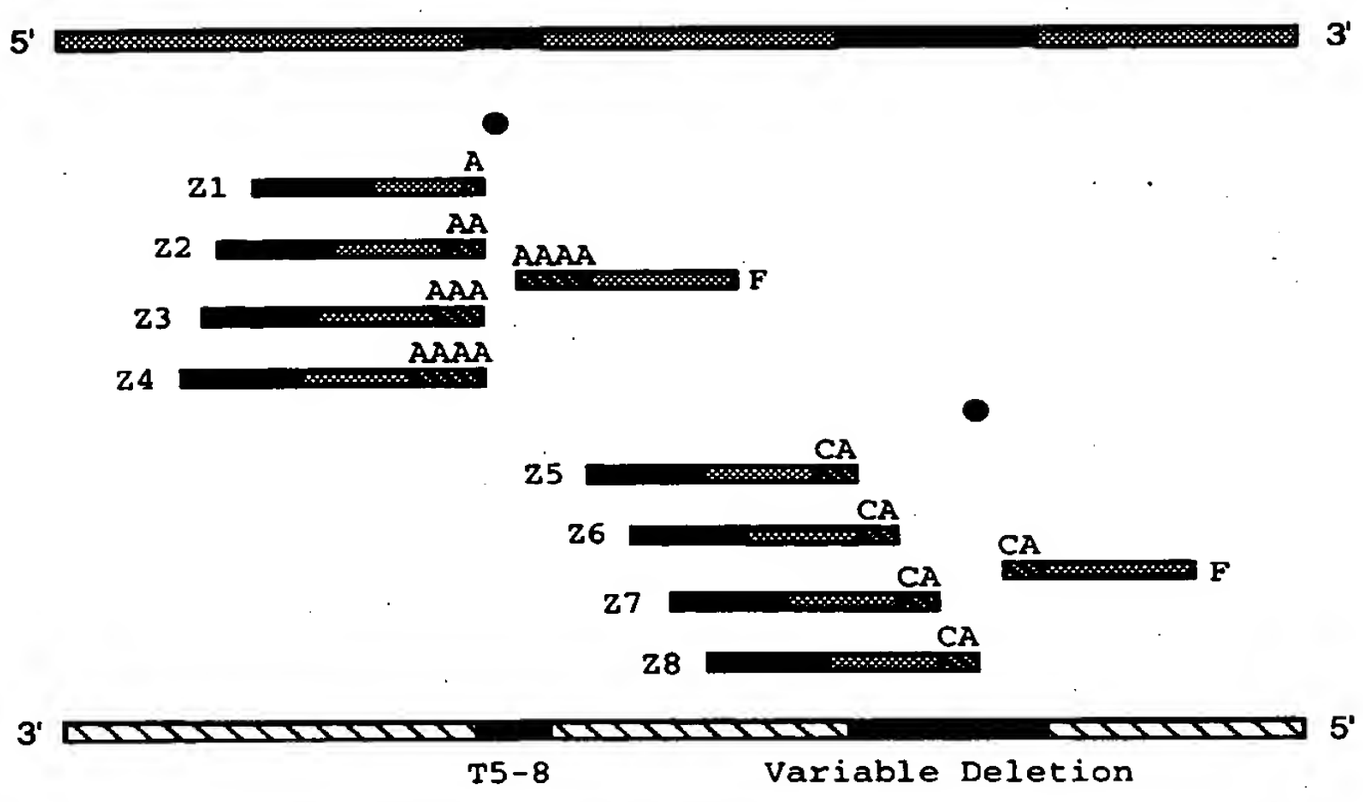
FIG. 5

PCR/ LDR : Insertions and Deletions

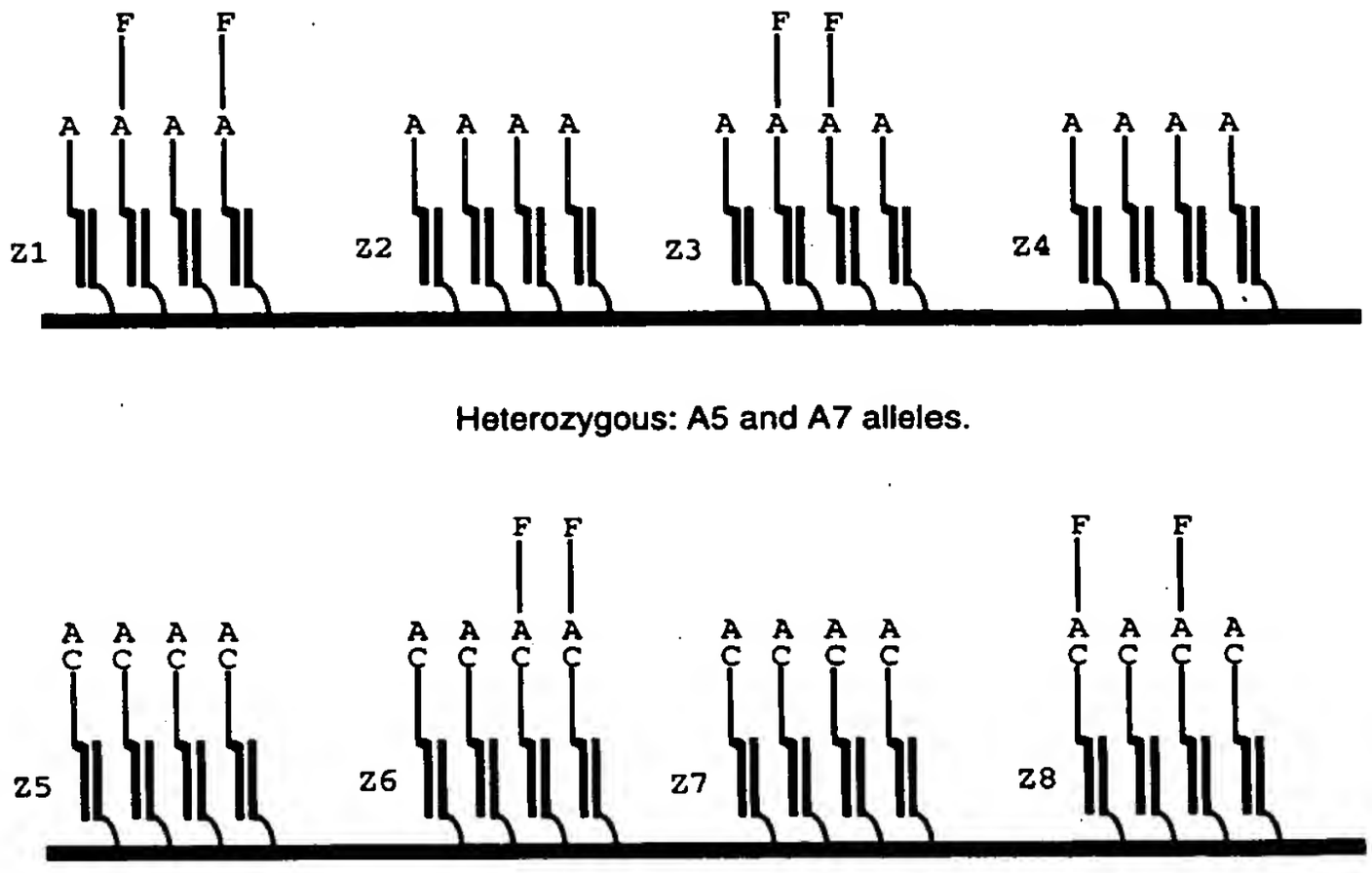
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



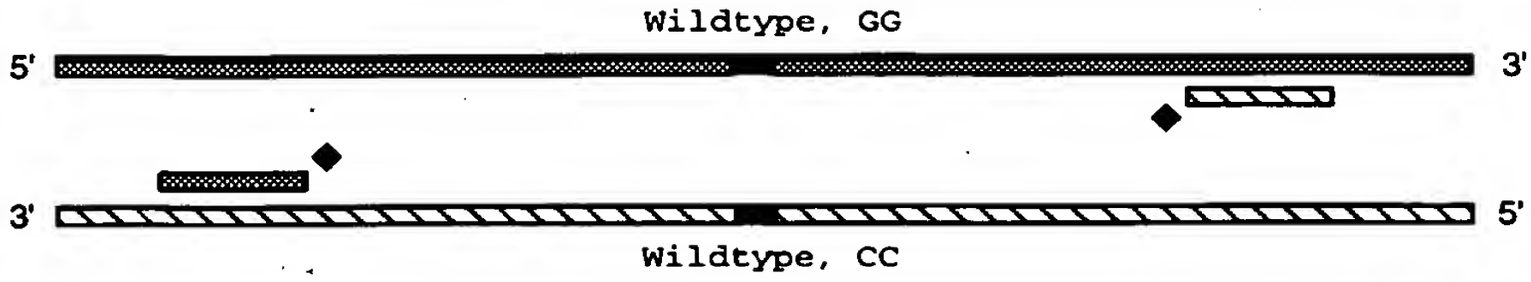
Heterozygous: A5 and A7 alleles.

Heterozygous: (CA)5 and (CA)3 alleles.

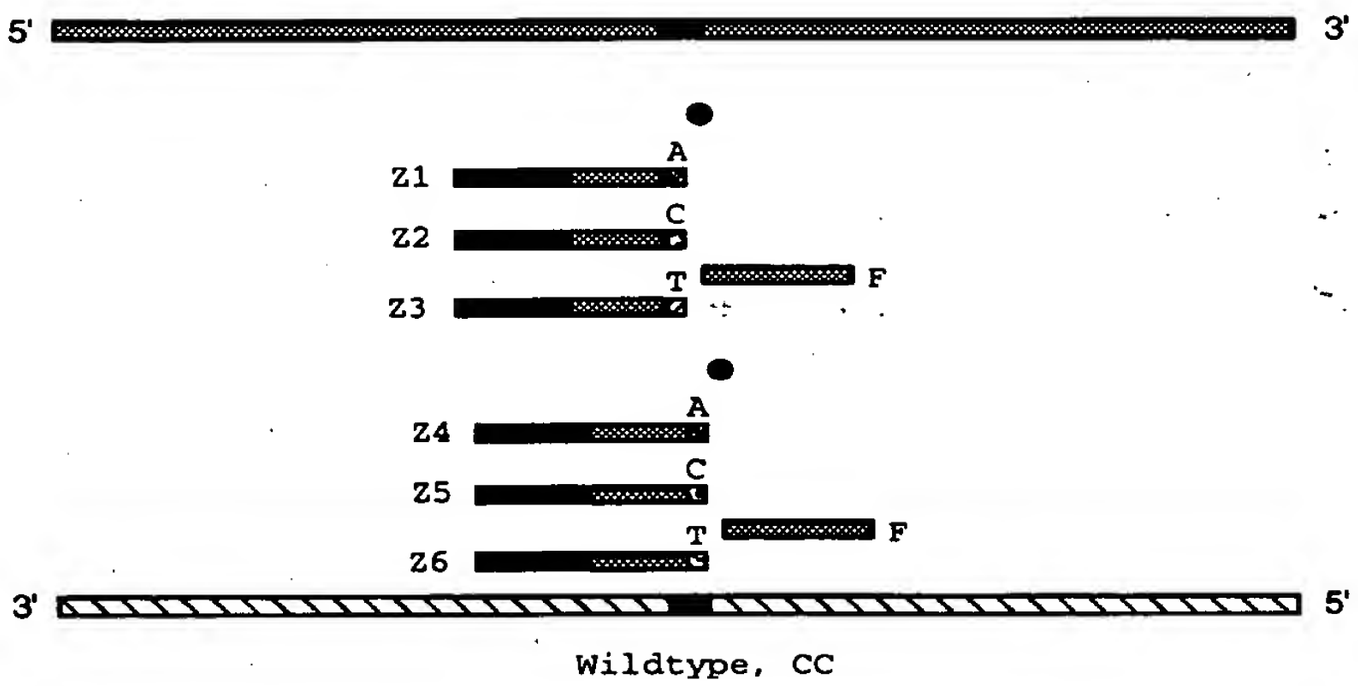
FIG. 6

PCR/ LDR : Adjacent alleles, cancer detection

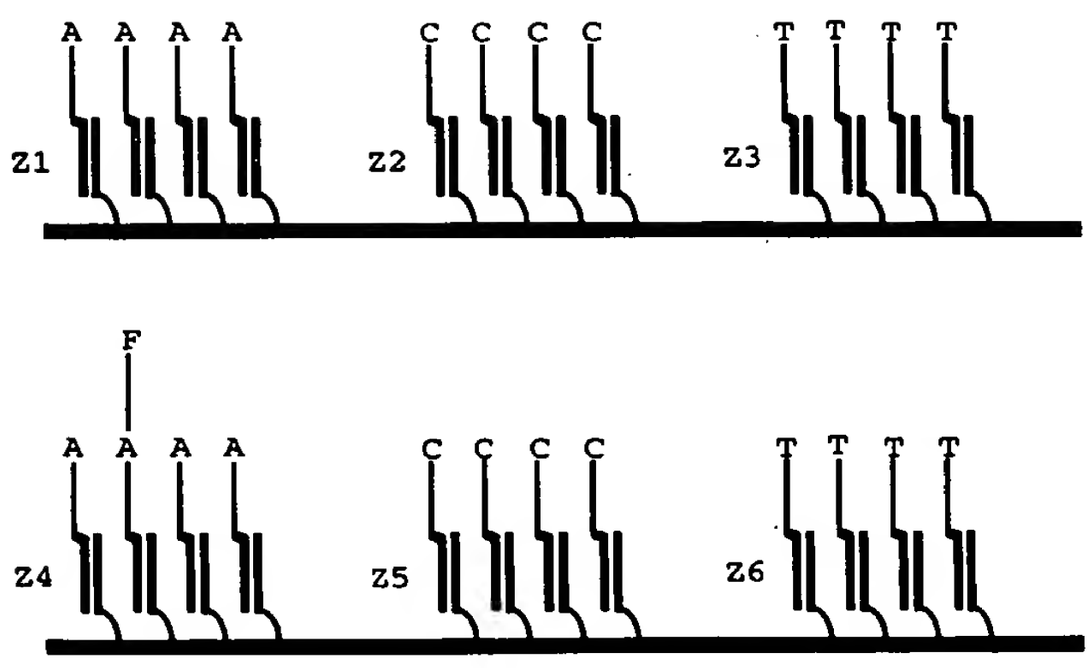
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.

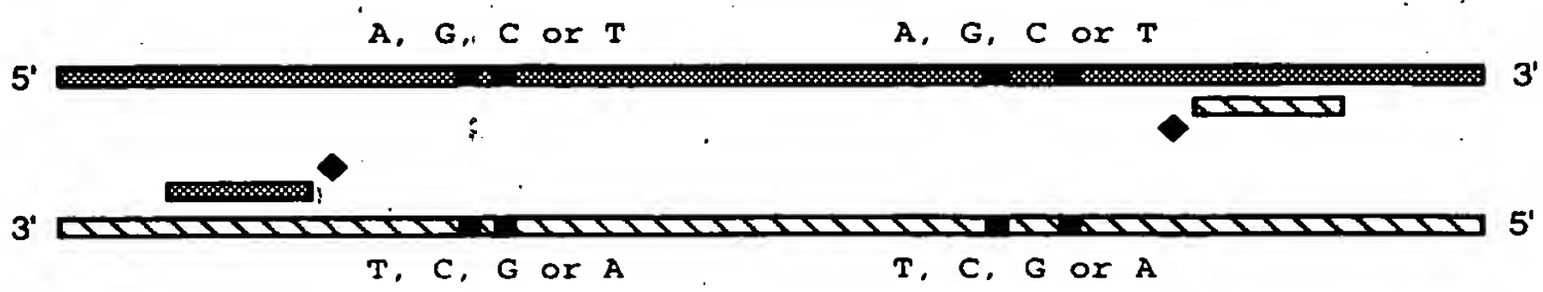


Gly to Asp mutation

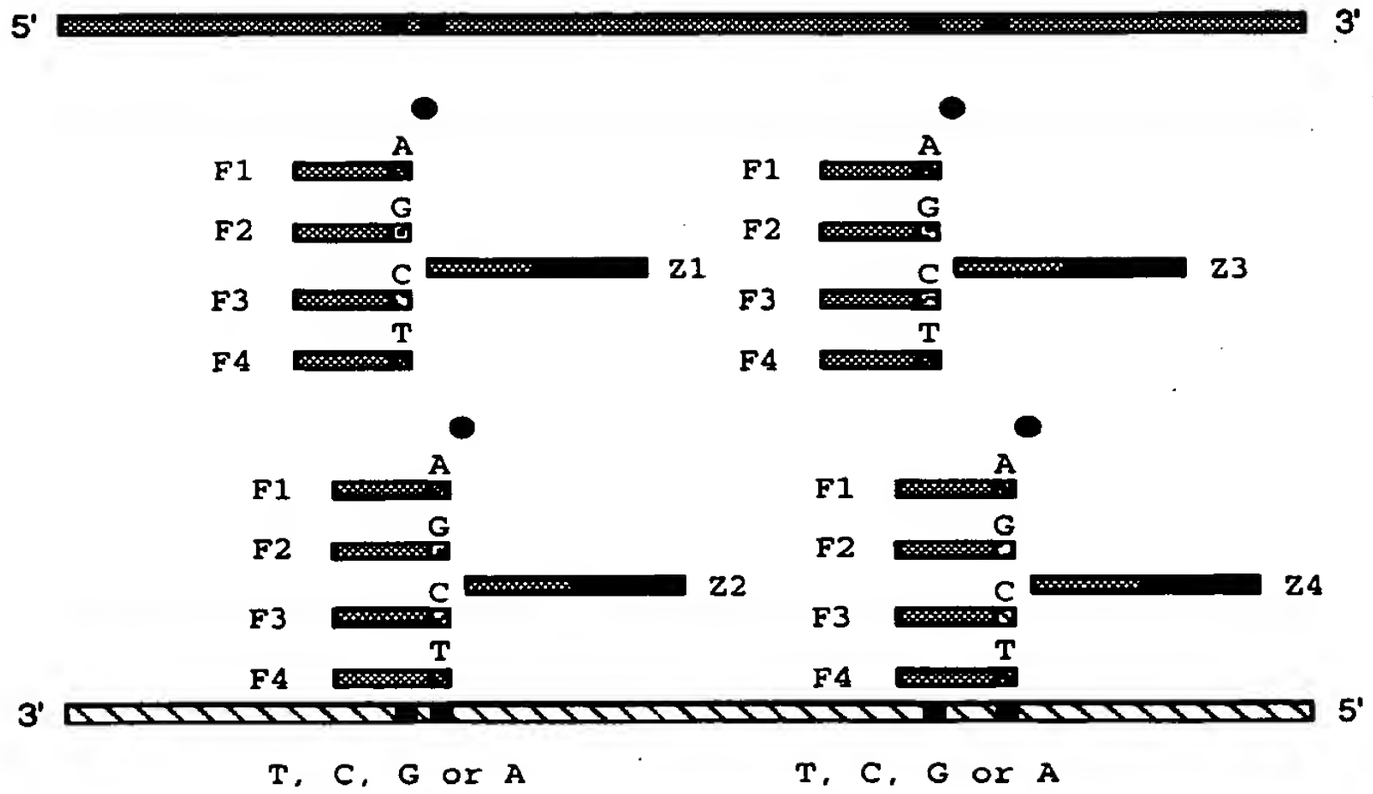
FIG. 7

PCR/ LDR : Nearby alleles

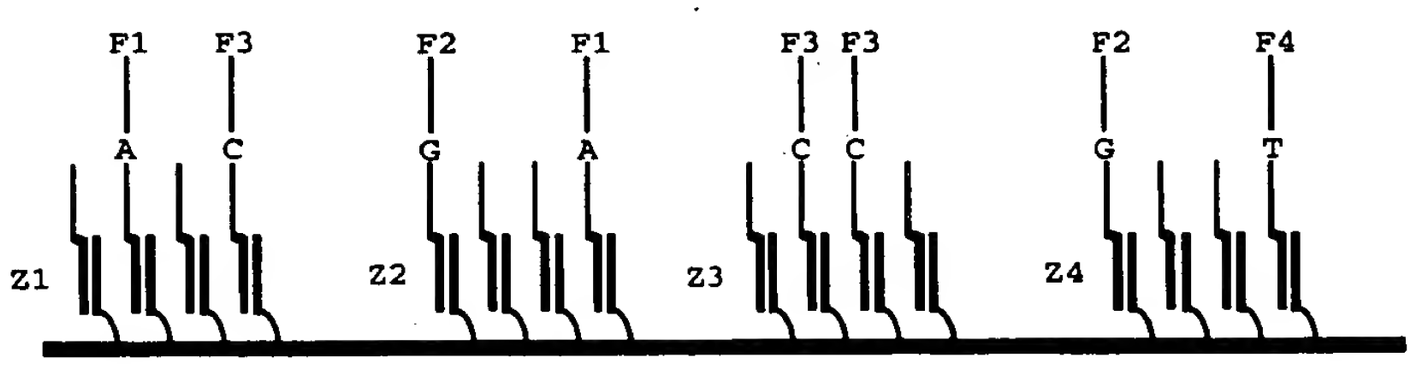
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A and C alleles.

Heterozygous: A and G alleles.

Homozygous: C allele.

Heterozygous: G and T alleles.

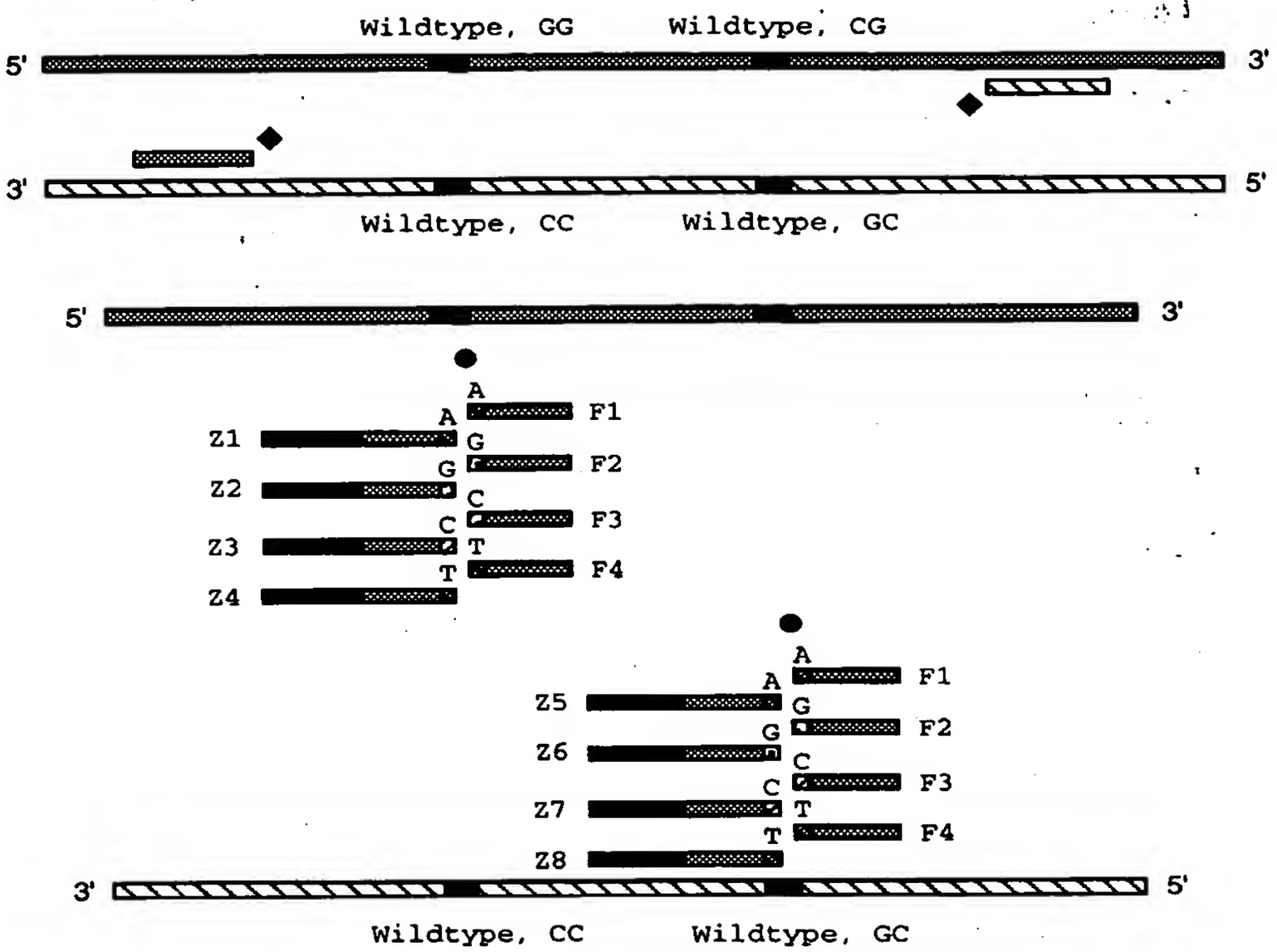
FIG. 8

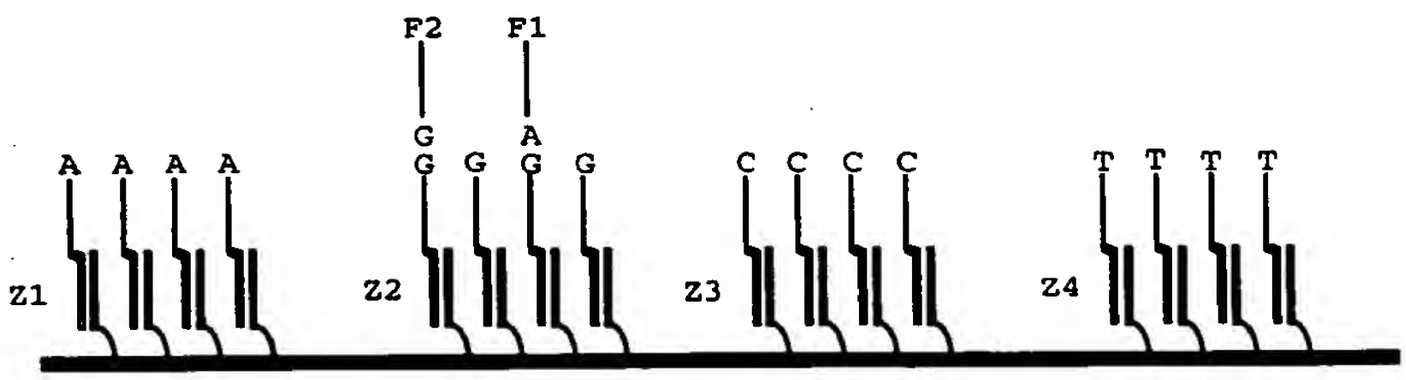
PCR/ LDR : Adjacent and Nearby alleles

1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆

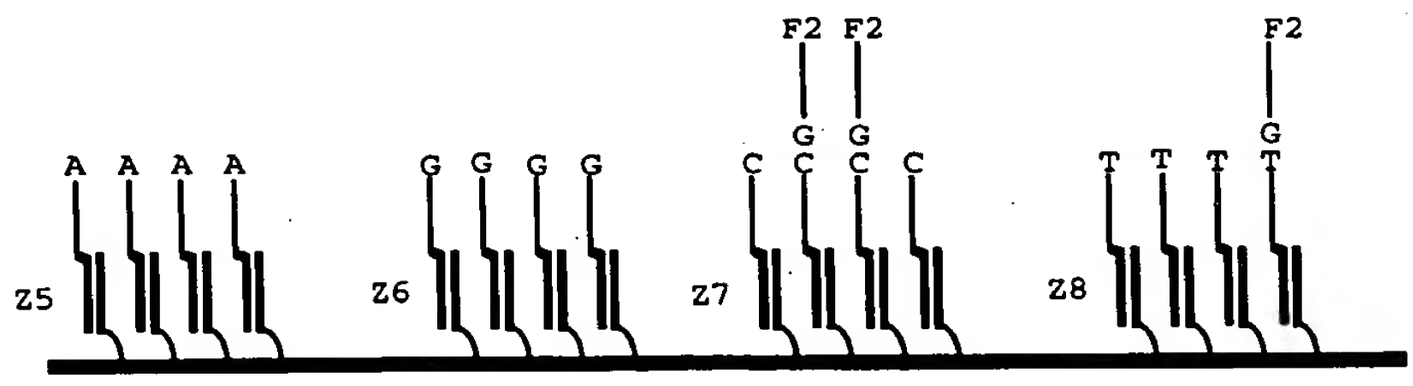
2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

3. Capture fluorescent products on addressable array and quantify each allele.





Heterozygous: Gly and Glu alleles.



Heterozygous: Arg and Trp alleles.

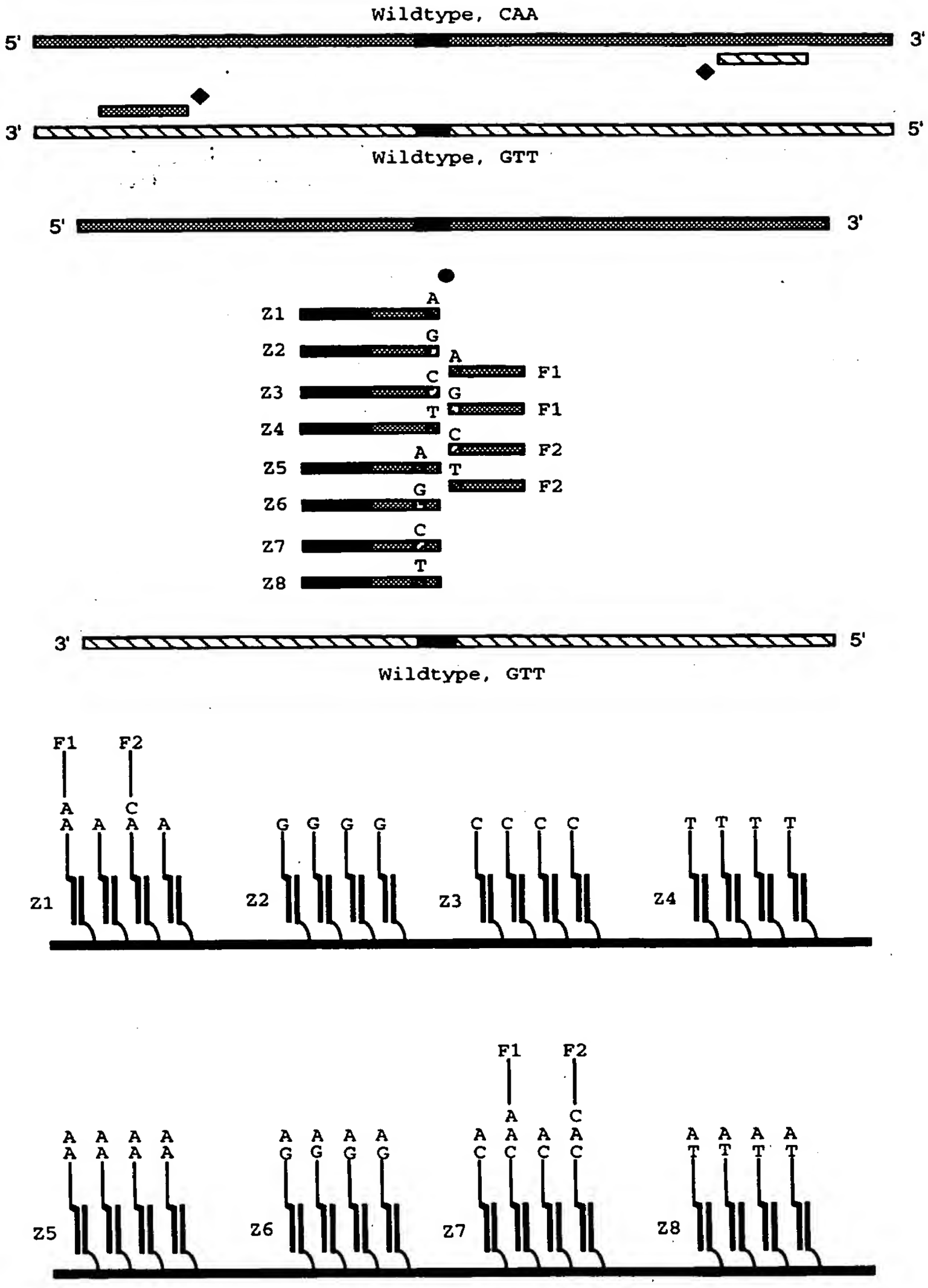
FIG. 9

PCR/ LDR : All alleles of a single codon

1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆

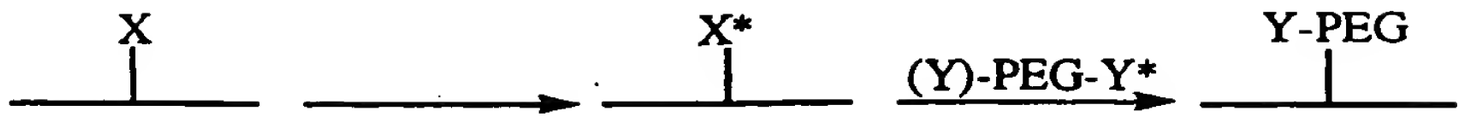
2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

3. Capture fluorescent products on addressable array and quantify each allele.



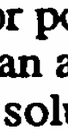
Heterozygous: Gln and His alleles.

FIG. 10



X,Y = –OH, –CO_2H, –NH_2
(Y) = W–NH–

X*, Y* = –O(C=O)Z, –O(C=S)Z, –CO_2H, –(C=O)Z, –NH_2, –N=C=O

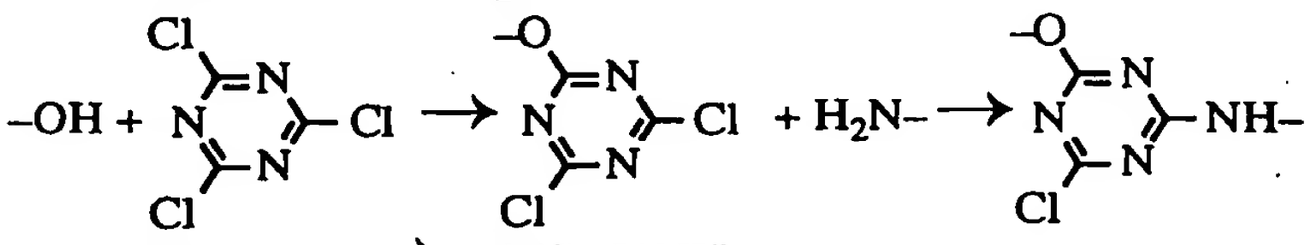
W = protecting group, e.g. Boc, Fmoc
Z = activating group, e.g. imidazole (Im), *p*-nitrophenol (OPnp), hydroxysuccinimide (OSu), pentafluorophenol (OPfp)
PEG = oligo or poly(ethylene glycol), backbone $(CH_2CH_2O)_n$ n = 6 to 200 (can also be grown by anionic polymerization with )
WSC = water soluble carbodiimide

Functional group transformations/activation (as needed), X → X*, Y → Y*

–OH ⟶ $–O(CH_2)_nCO_2H$ n = 1, 2
–OH ⟶ $–O(C=O)NHCH_2CO_2H$
–OH ⟶ $–O(C=O)CH_2NH_2$
–OH ⟶ –O(C=O)Im
–OH ⟶ $–O(C=S)SCH_2(C=O)NH_2$
$–CO_2H$ ⟶ $–(C=O)NH(CH_2)_nNH_2$ n = 2,6
$–CO_2H$ ⟶ –(C=O)Z
$–NH_2$ ⟶ $–NH(C=O)(CH_2)_nCO_2H$ n = 2, 3

Covalent linkage, X* + Y*

$–CO_2H + H_2N– + WSC + HOSu$ ⟶ –(C=O)NH–
$–OH + Im(C=O)Im + H_2N–$ ⟶ –O(C=O)NH–



–OH + O=C=N– ⟶ –O(C=O)NH–
$–O(C=S)SCH_2(C=O)NH_2 + H_2N–$ ⟶ –O(C=S)NH–
–OH +  + HO– ⟶ $–OCH_2CH(OH)CH_2O–$ (+ $H_2NH–$) (NH–)
–OH ⟶ $–OCH_2(C=O)H + H_2N– + NaCNBH_3$ ⟶ $–OCH_2CH_2NH–$

FIG. 11

FIG. 12A

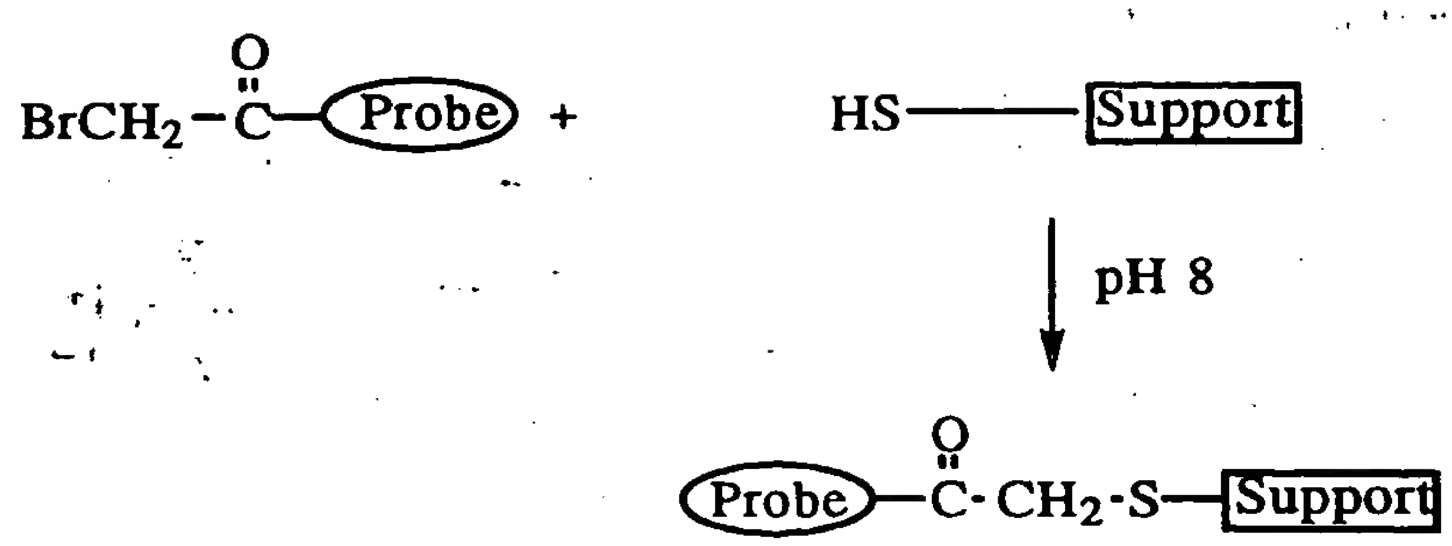


FIG. 12B

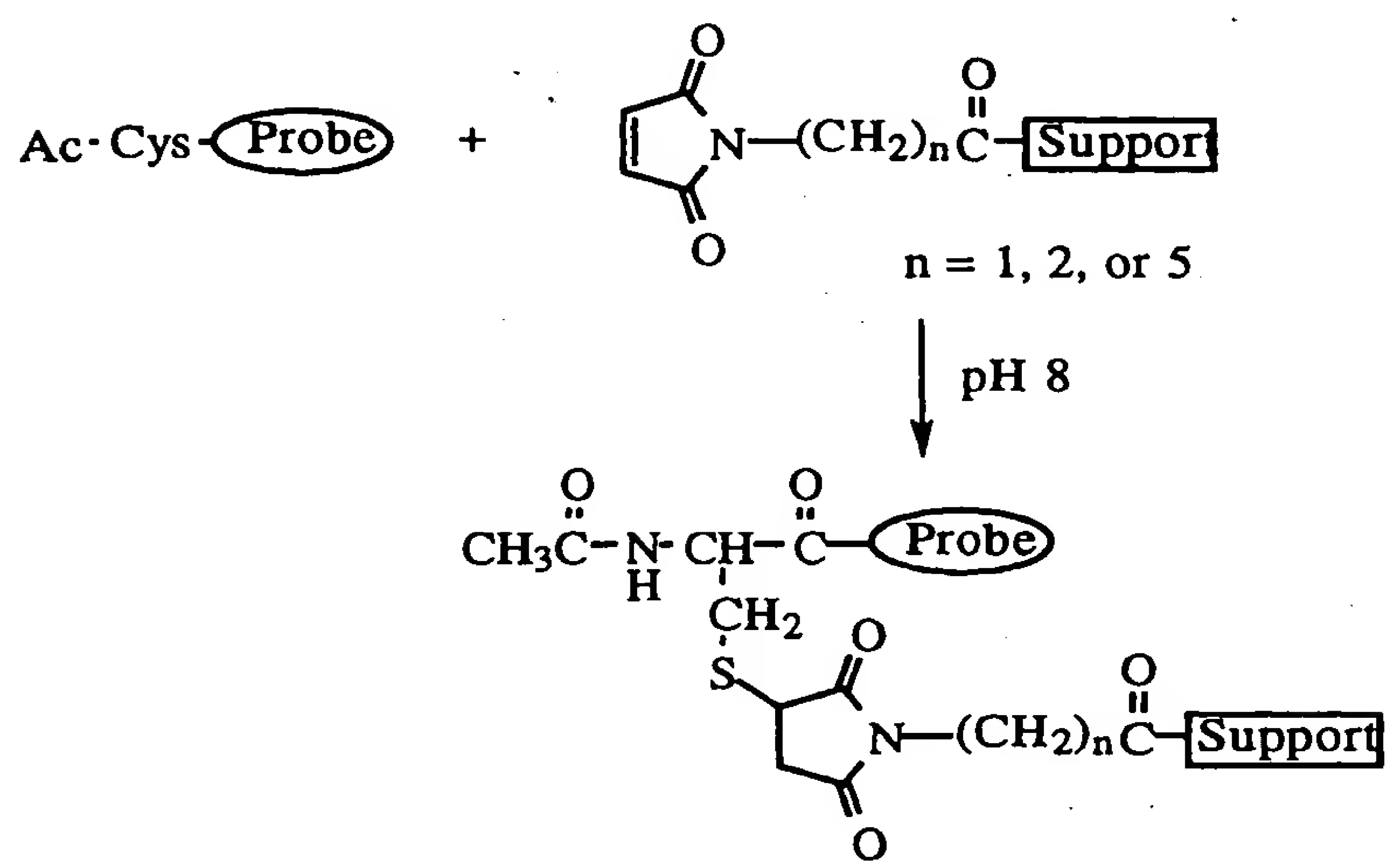
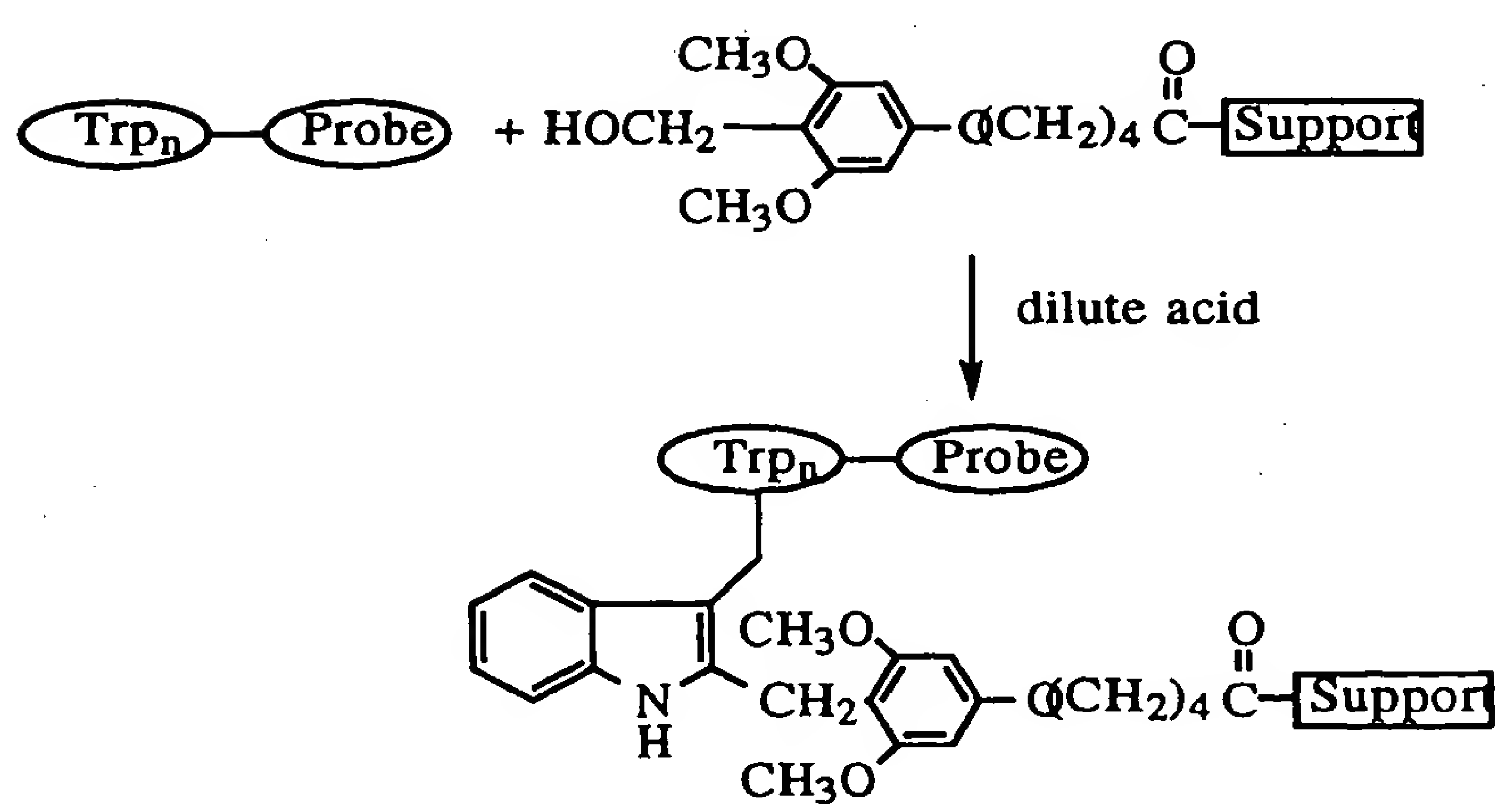


FIG. 12C



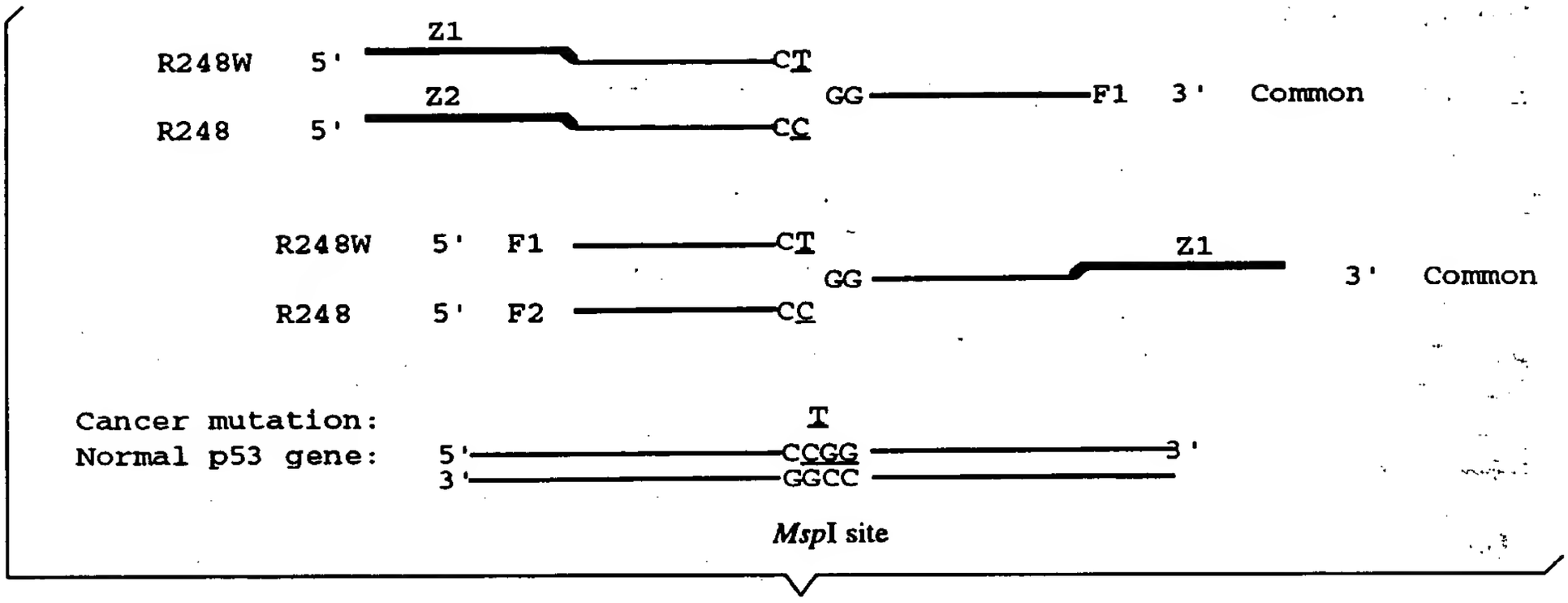


FIG. 13A

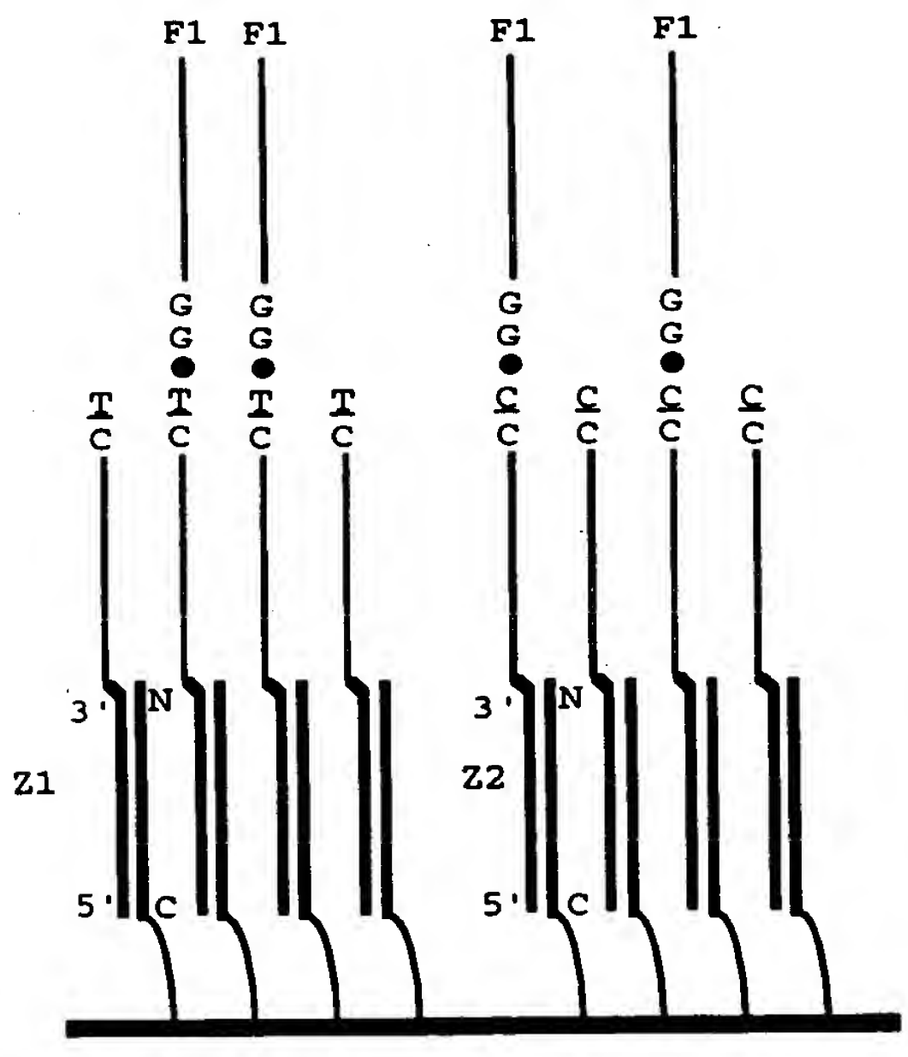


FIG. 13B

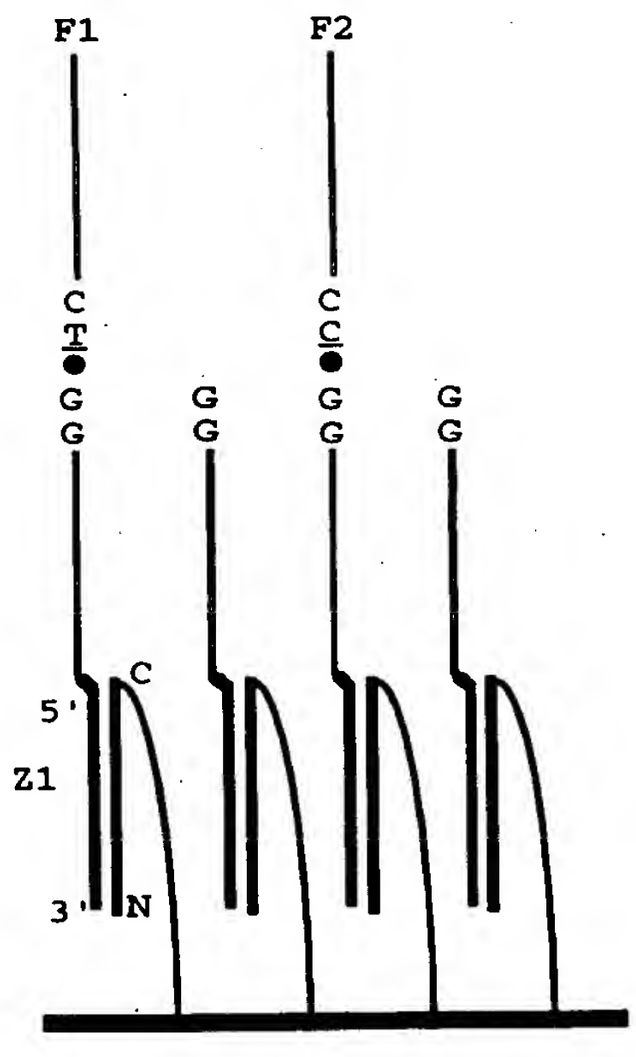


FIG. 13C

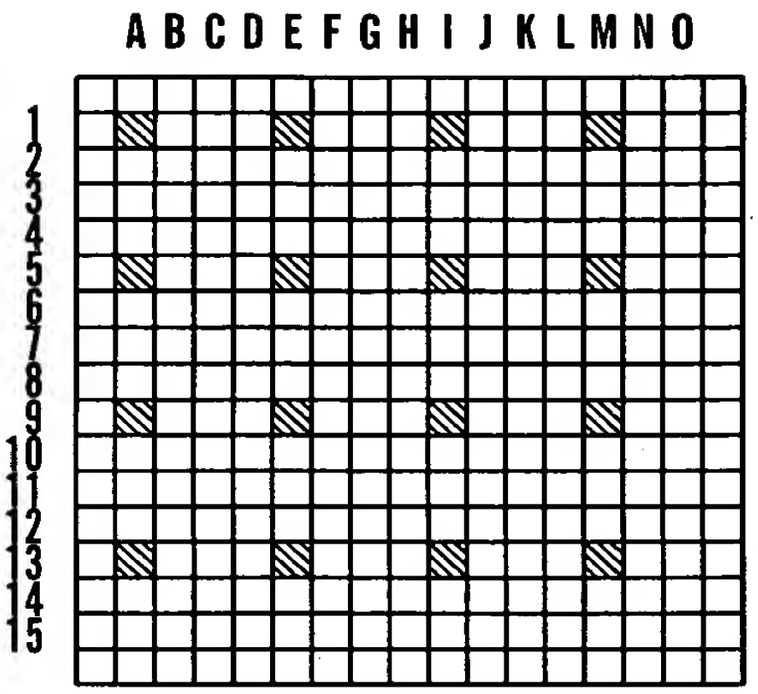


FIG. 14A

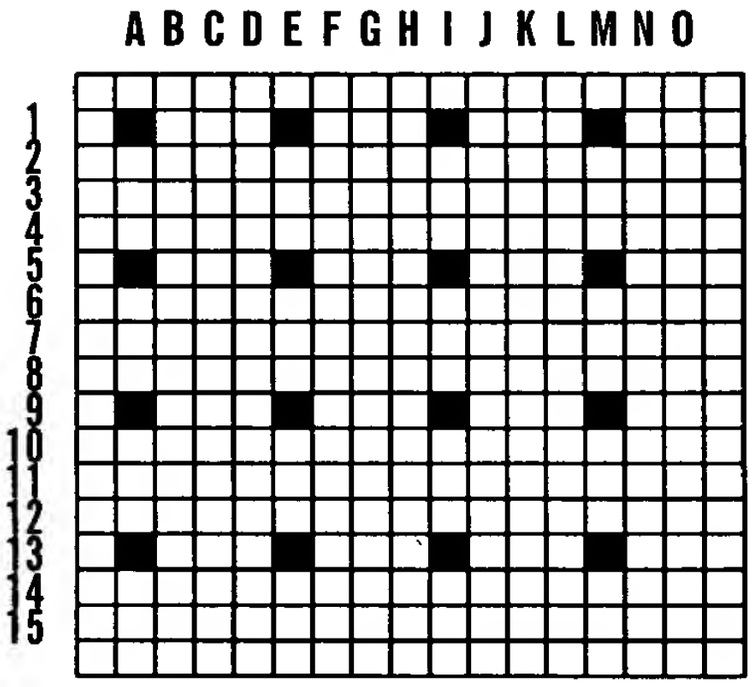


FIG. 14B

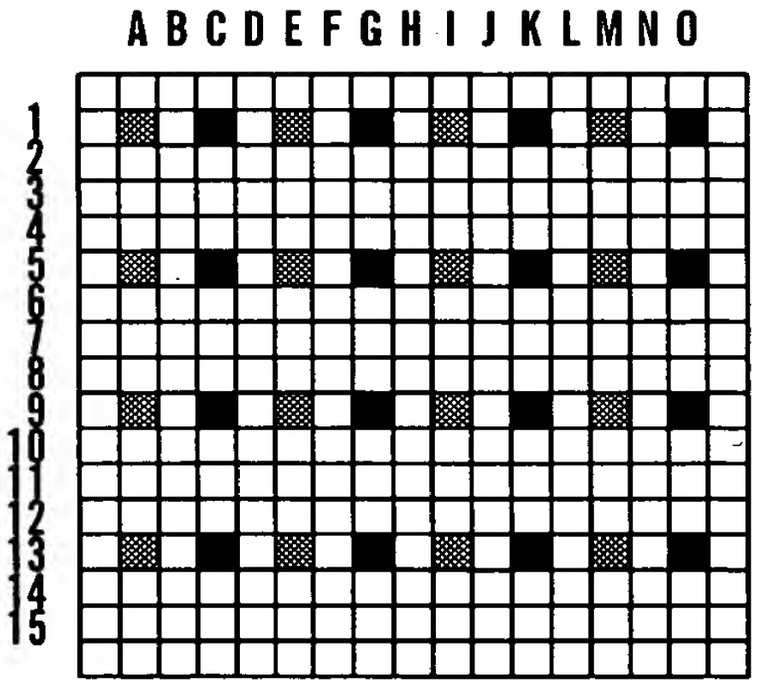


FIG. 14C

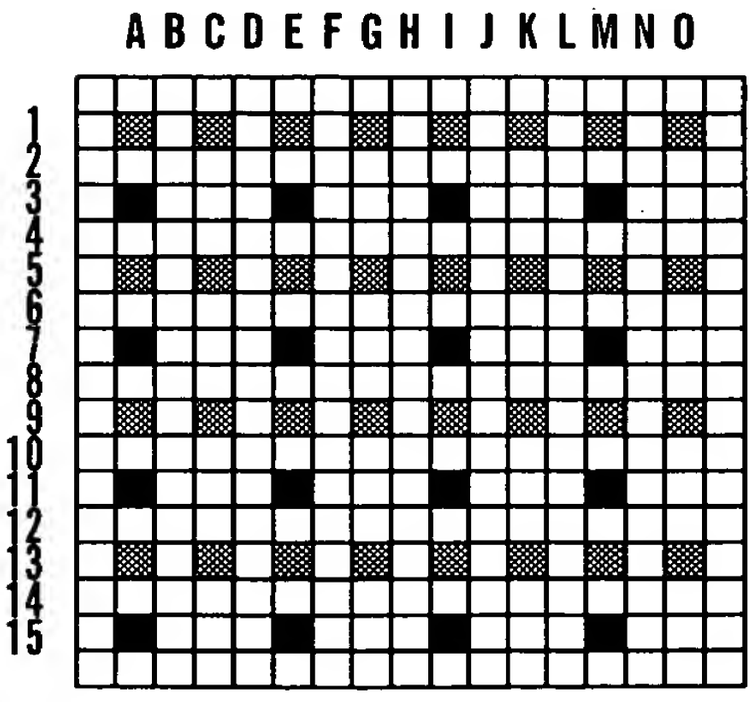


FIG. 14D

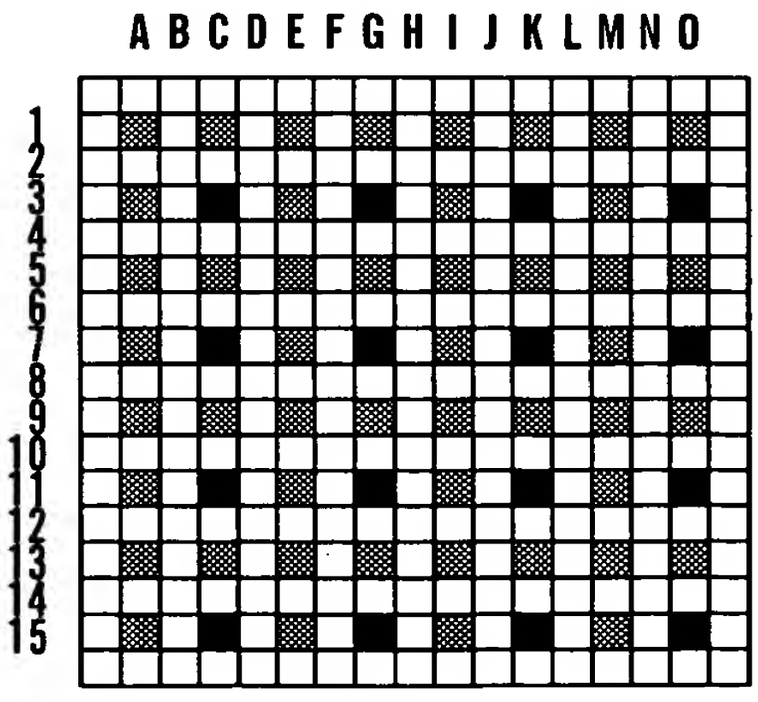


FIG. 14E

FIG. 15A

1st addition of unique 24mers.

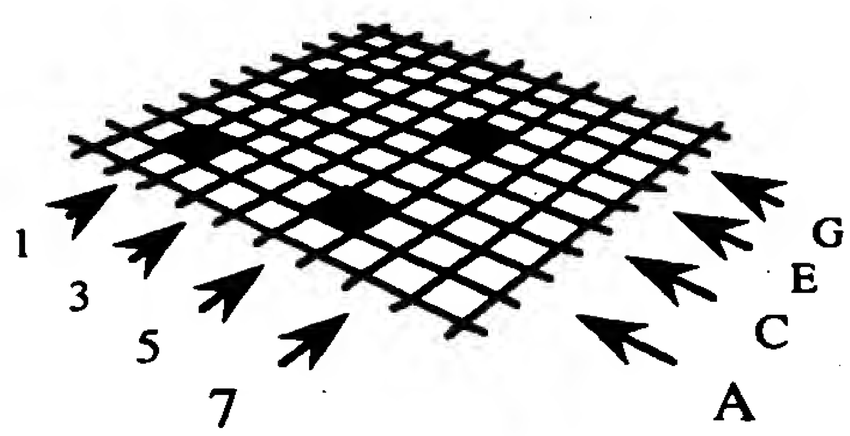


FIG. 15B

2nd addition of unique 24mers.

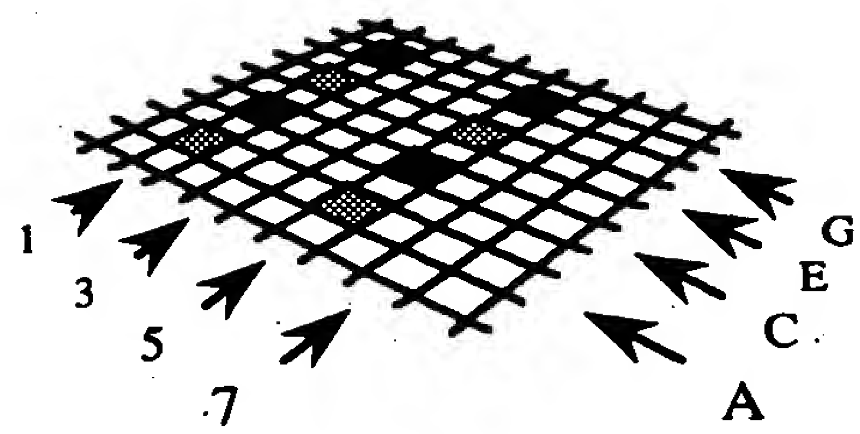


FIG. 15C

3rd addition of unique 24mers.

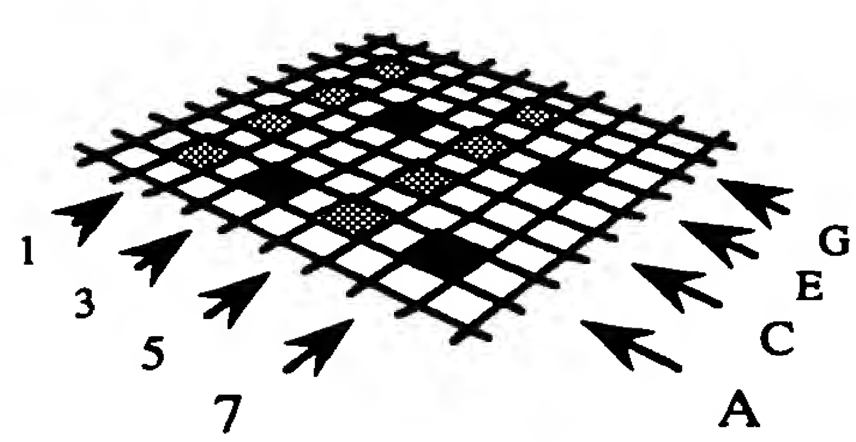
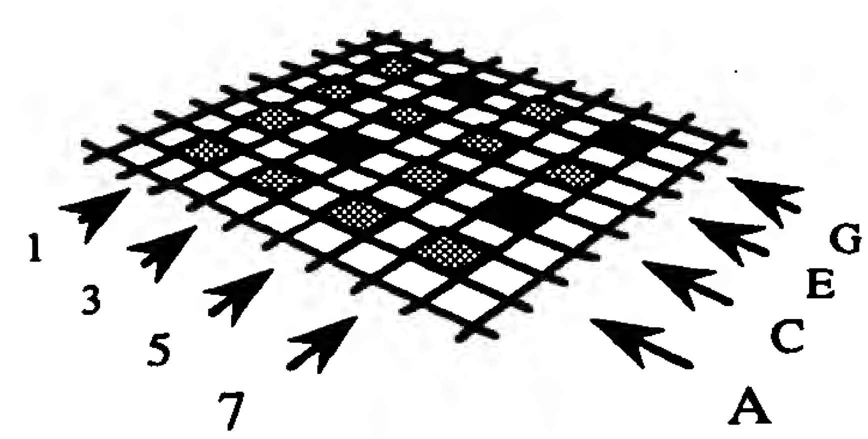


FIG. 15D

4th addition of unique 24mers.



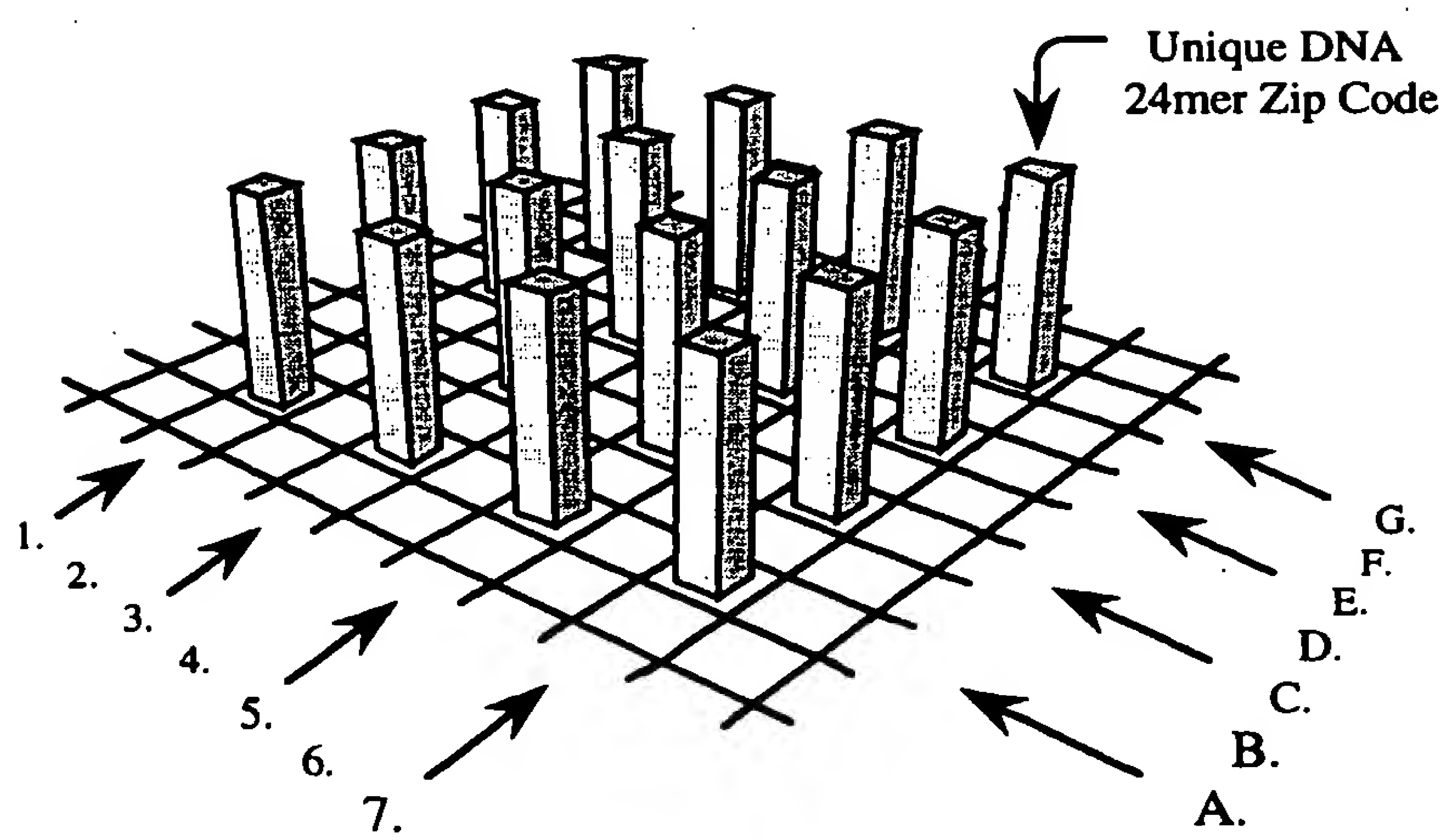


FIG. 15E

FIG. 16A

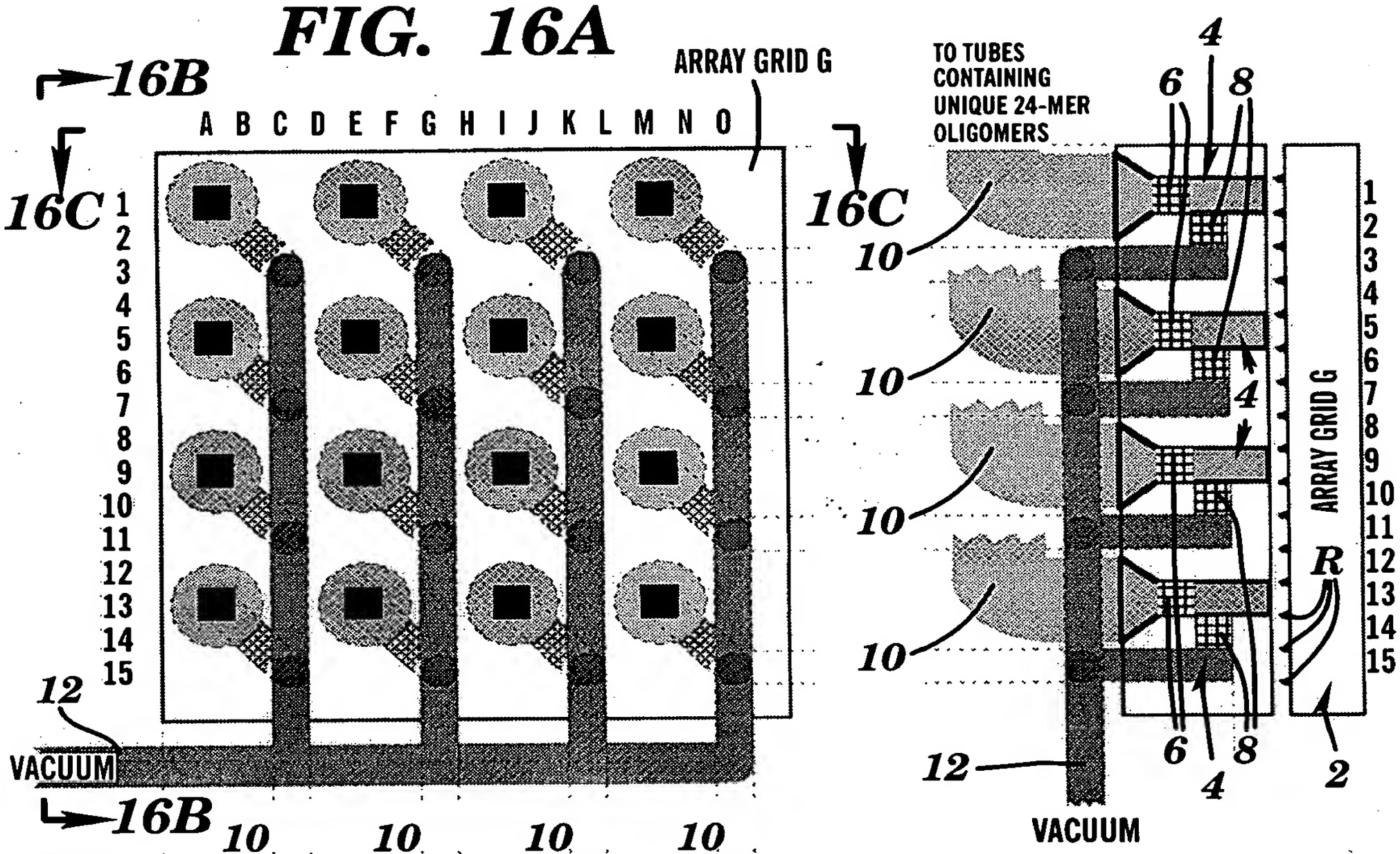


FIG. 16B

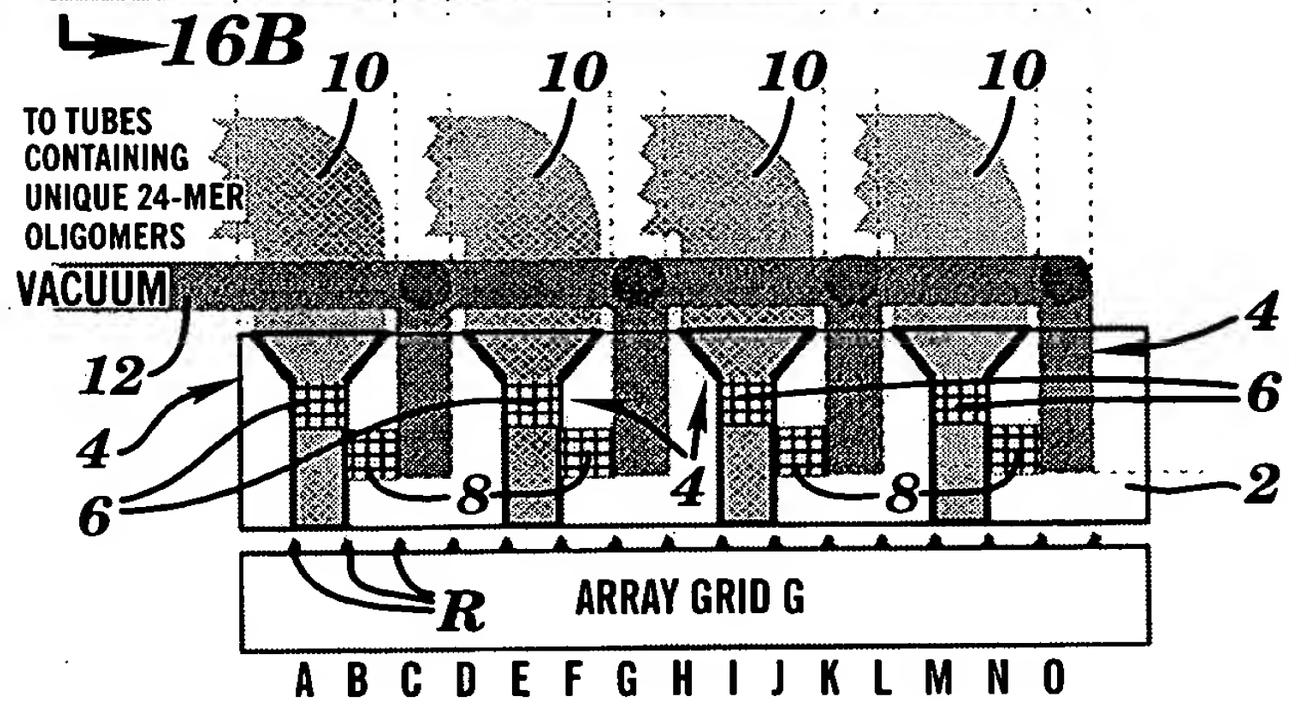


FIG. 16C

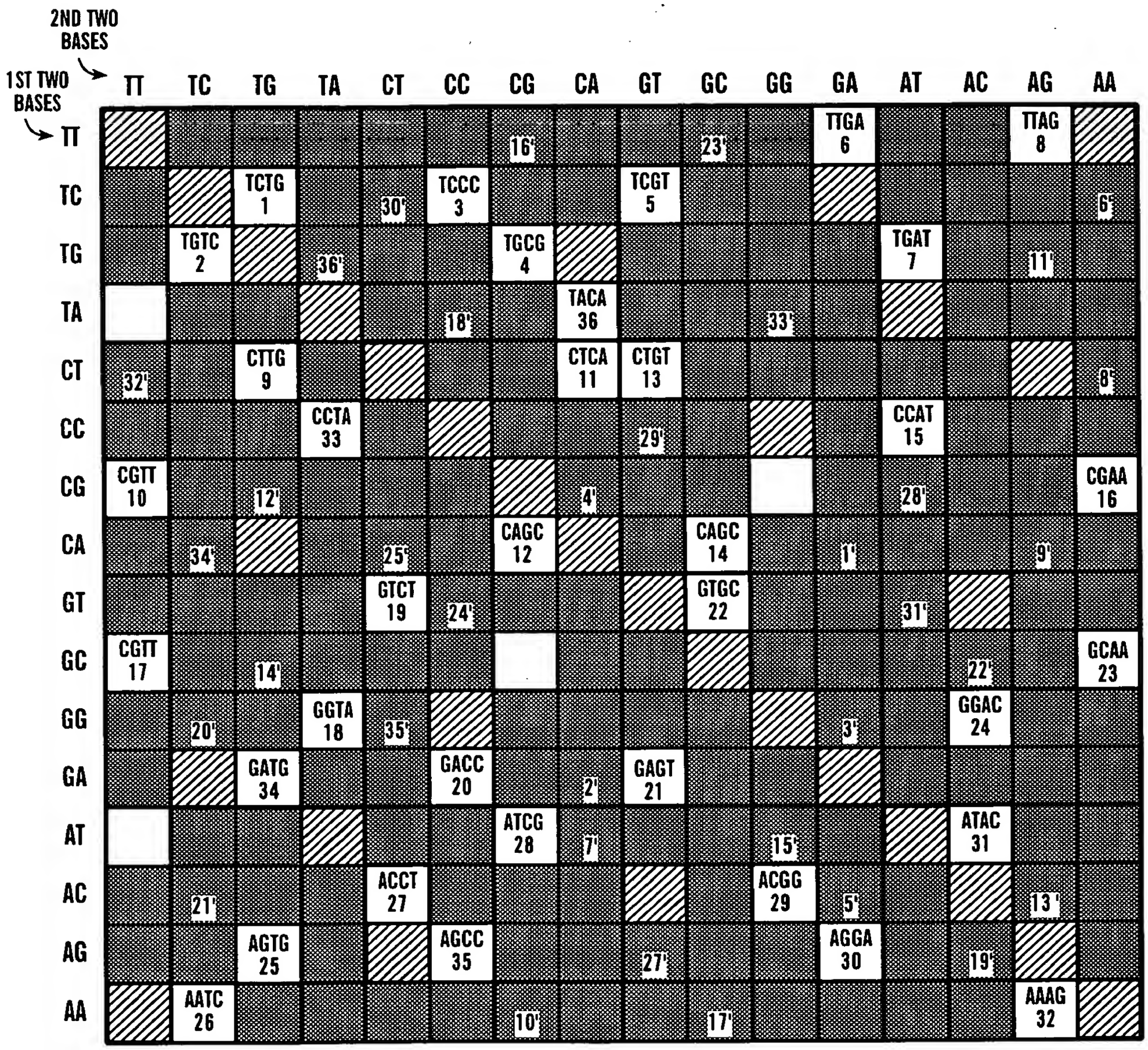

2ND TWO BASES
1ST TWO BASES
TT TC TG TA CT CC CG CA GT GC GG GA AT AC AG AA
TT
TC
TG
TA
CT
CC
CG
CA
GT
GC
GG
GA
AT
AC
AG
AA
TTGA 6
TTAG 8
16'
23'
TCTG 1
TCCC 3
TCGT 5
30'
6'
TGTC 2
TGCG 4
TGAT 7
36'
11'
TACA 36
18'
33'
CTTG 9
CTCA 11
CTGT 13
32'
8'
CCTA 33
CCAT 15
29'
CGTT 10
CGAA 16
12'
4'
28'
CAGC 12
CAGC 14
34'
25'
1'
9'
GTCT 19
GTGC 22
24'
31'
CGTT 17
GCAA 23
14'
22'
GGTA 18
GGAC 24
20'
35'
3'
GATG 34
GACC 20
GAGT 21
2'
ATCG 28
ATAC 31
7'
15'
ACCT 27
ACGG 29
21'
5'
13'
AGTG 25
AGCC 35
AGGA 30
27'
19'
AATC 26
AAAG 32
10'
17'

FIG. 17

1st Tetramer addition
(columns)

1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5

FIG. 18A

2nd Tetramer addition
(rows)

6	6	6	6	6
5	5	5	5	5
4	4	4	4	4
3	3	3	3	3
2	2	2	2	2

FIG. 18B

3rd Tetramer addition
(columns)

3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1

FIG. 18C

4th Tetramer addition
(rows)

2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5
4	4	4	4	4

FIG. 18D

5th Tetramer addition
(columns)

6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4

FIG. 18E

6th Tetramer addition
(rows)

3	3	3	3	3
2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5

FIG. 18F

Addressable array with full length PNA 24mers

1-6-3-2-6-3	2-6-4-2-1-3	3-6-5-2-2-3	4-6-6-2-3-3	5-6-1-2-4-3
1-5-3-1-6-2	2-5-4-1-1-2	3-5-5-1-2-2	4-5-6-1-3-2	5-5-1-1-4-2
1-4-3-6-6-1	2-4-4-6-1-1	3-4-5-6-2-1	4-4-6-6-3-1	5-4-1-6-4-1
1-3-3-5-6-6	2-3-4-5-1-6	3-3-5-5-2-6	4-3-6-5-3-6	5-3-1-5-4-6
1-2-3-4-6-5	2-2-4-4-1-5	3-2-5-4-2-5	4-2-6-4-3-5	5-2-1-4-4-5

FIG. 18G

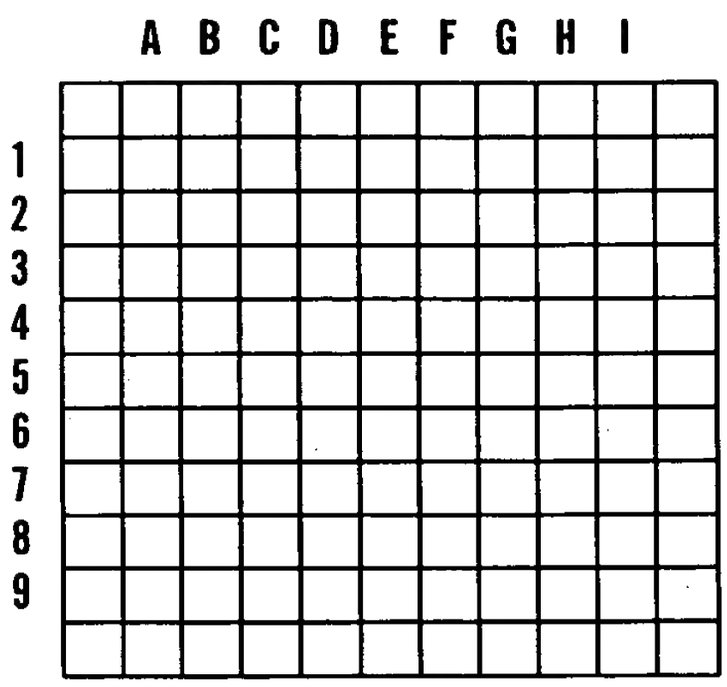


FIG. 19A

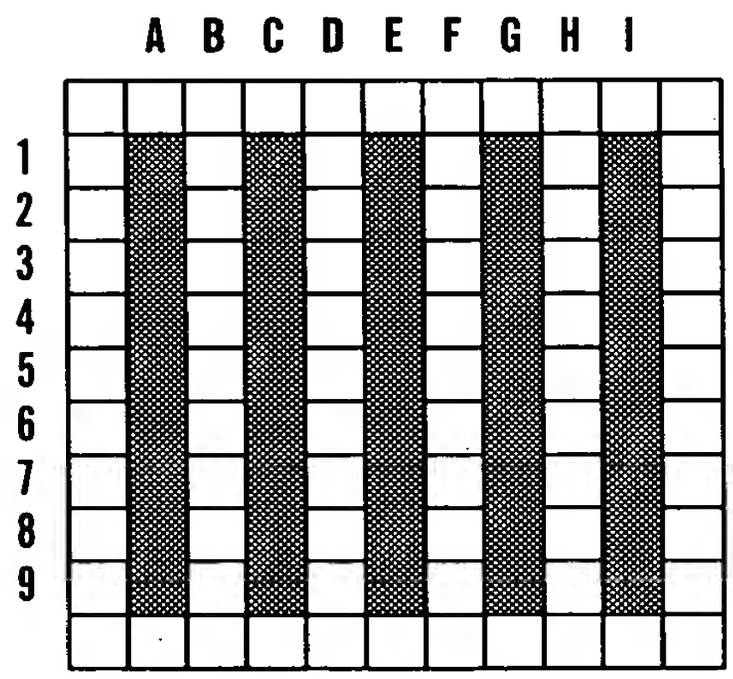


FIG. 19B

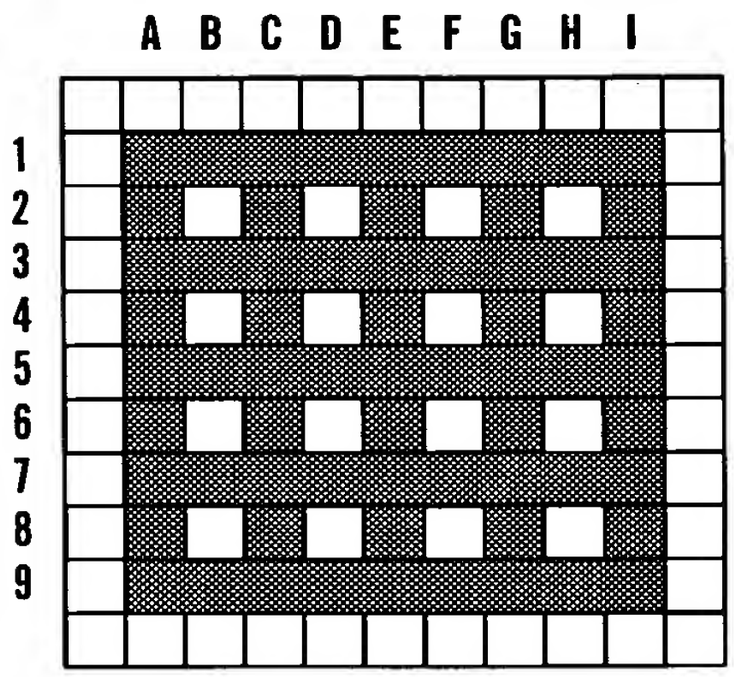


FIG. 19C

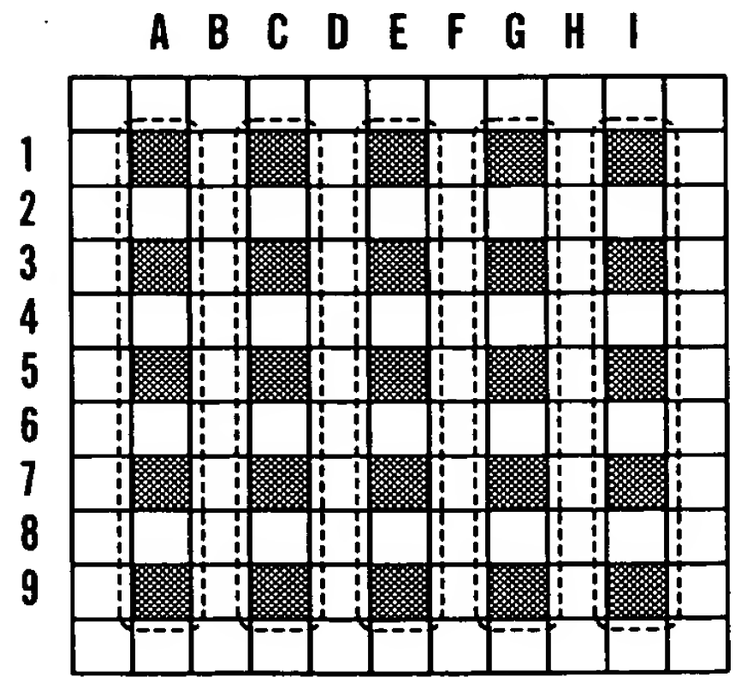


FIG. 19D

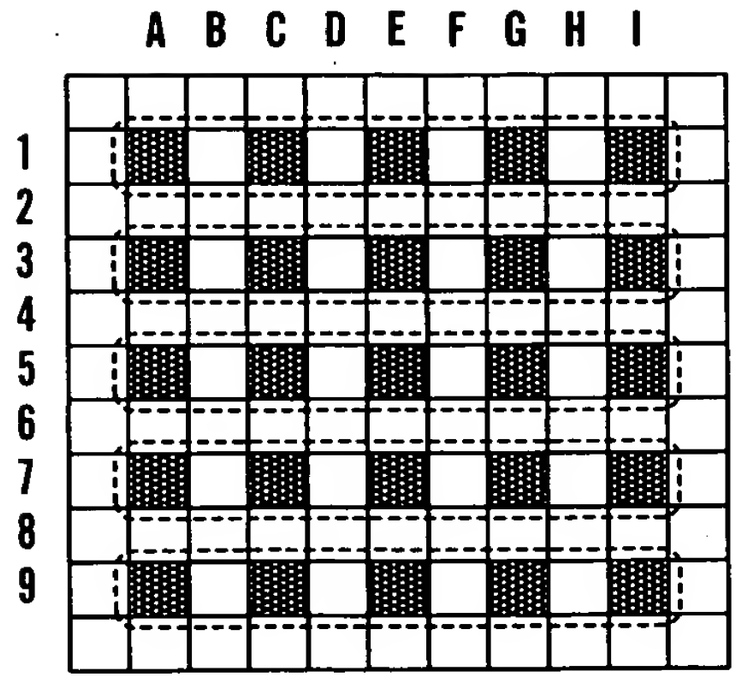


FIG. 19E

FIG. 20A

1st Tetramer additions
(columns)

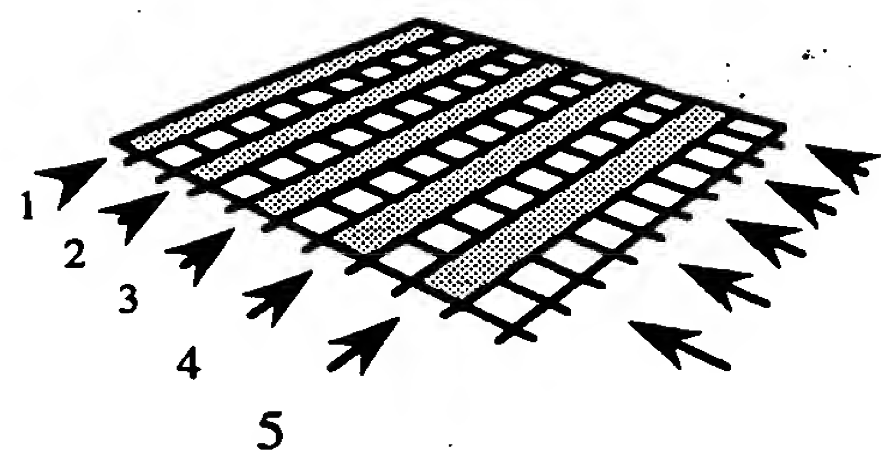
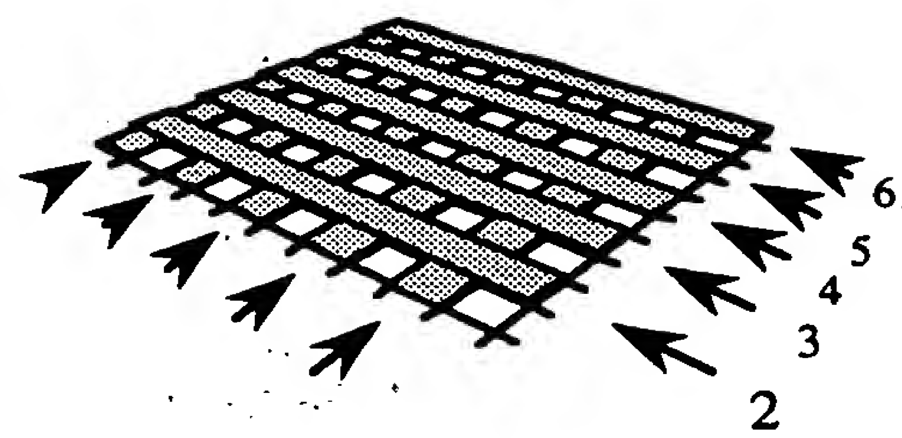


FIG. 20B

2nd Tetramer additions
(rows)



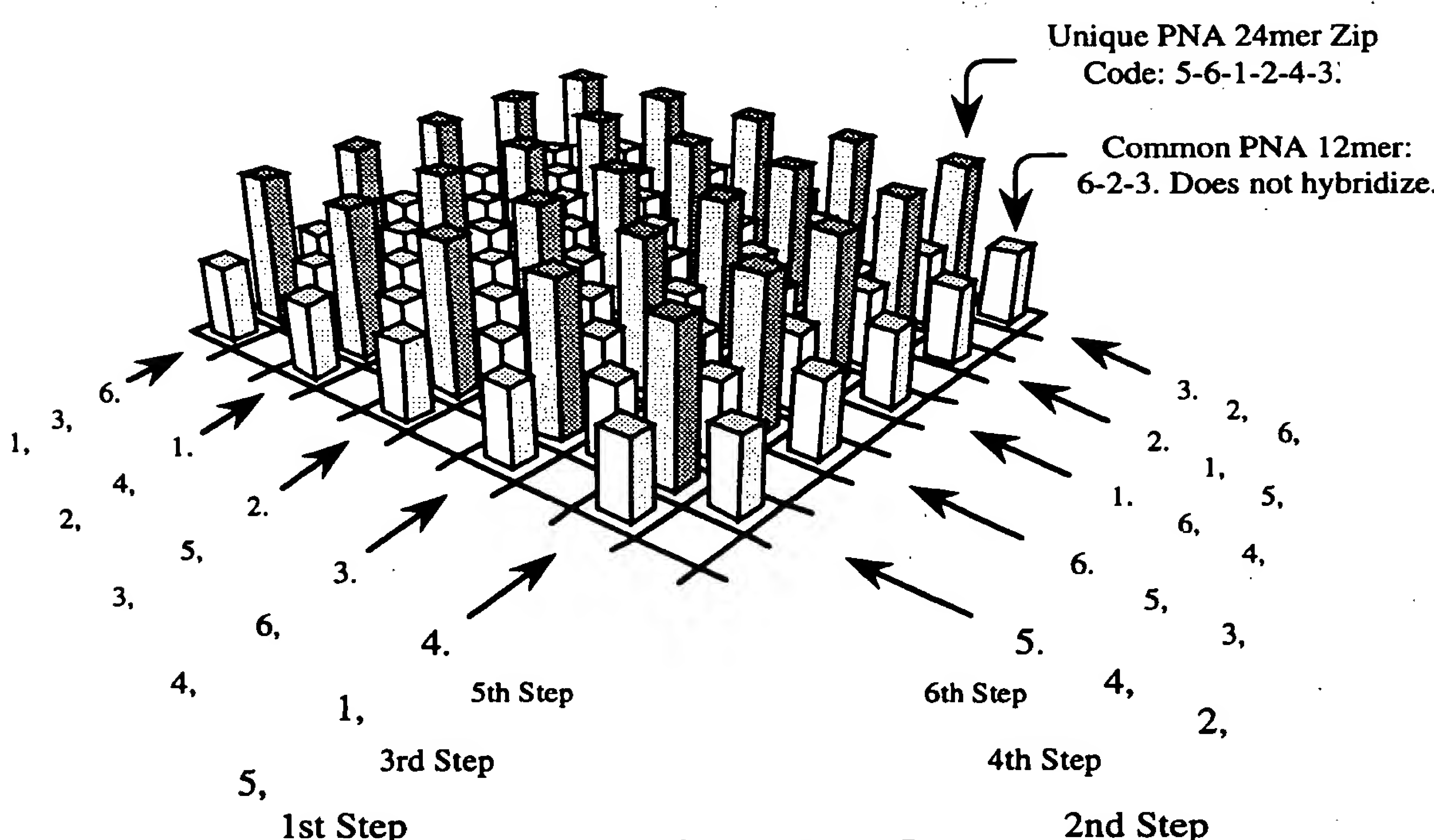


FIG. 20C

FIG. 21A

FIG. 21B

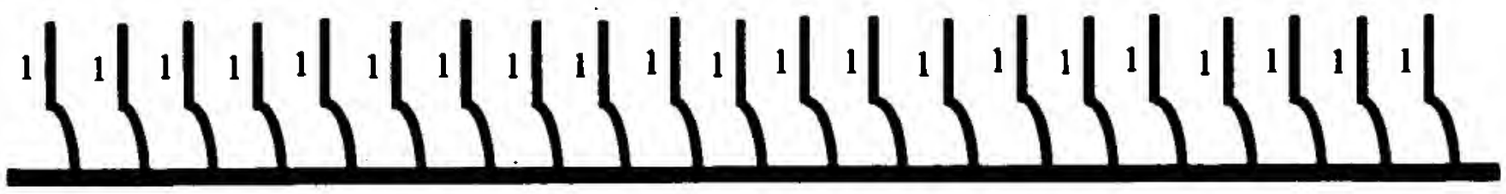


FIG. 21C

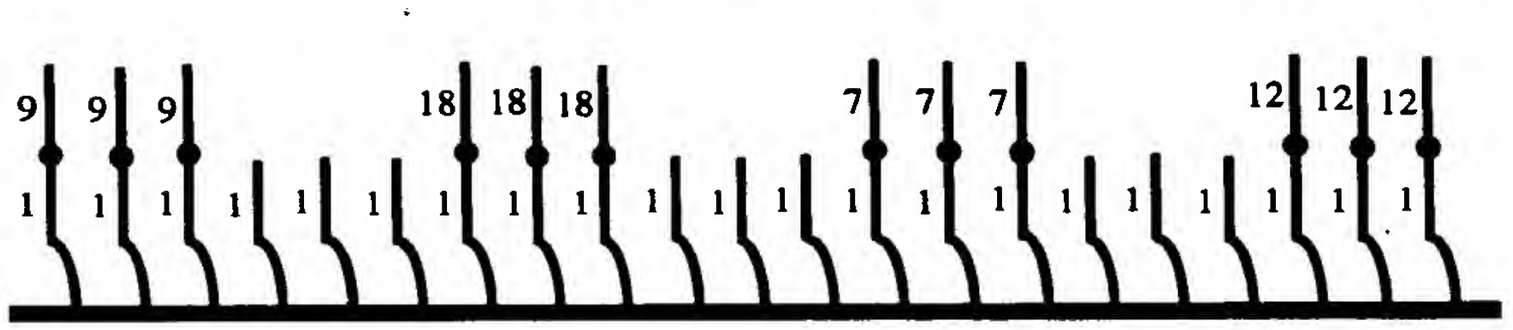


FIG. 21D

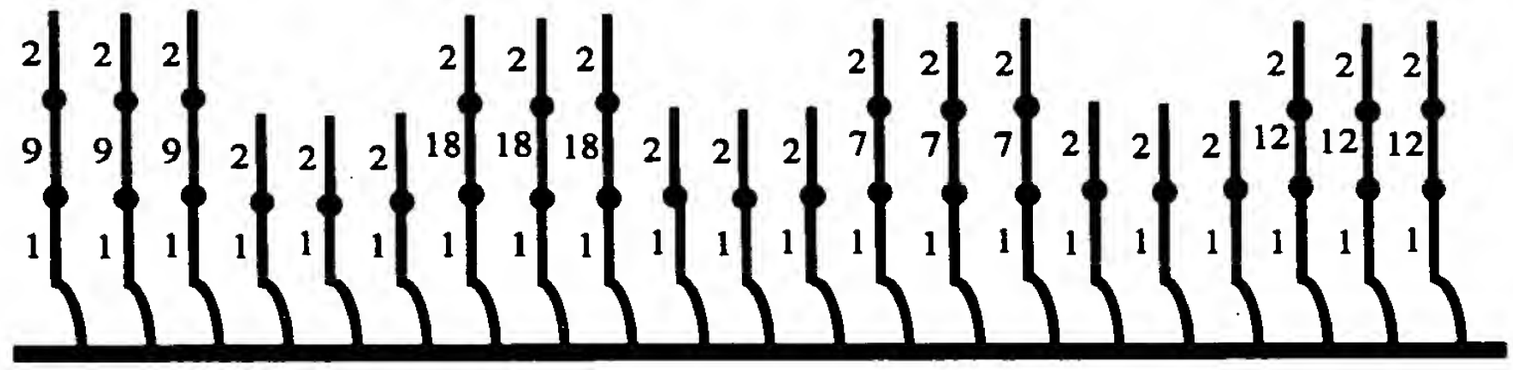


FIG. 21E

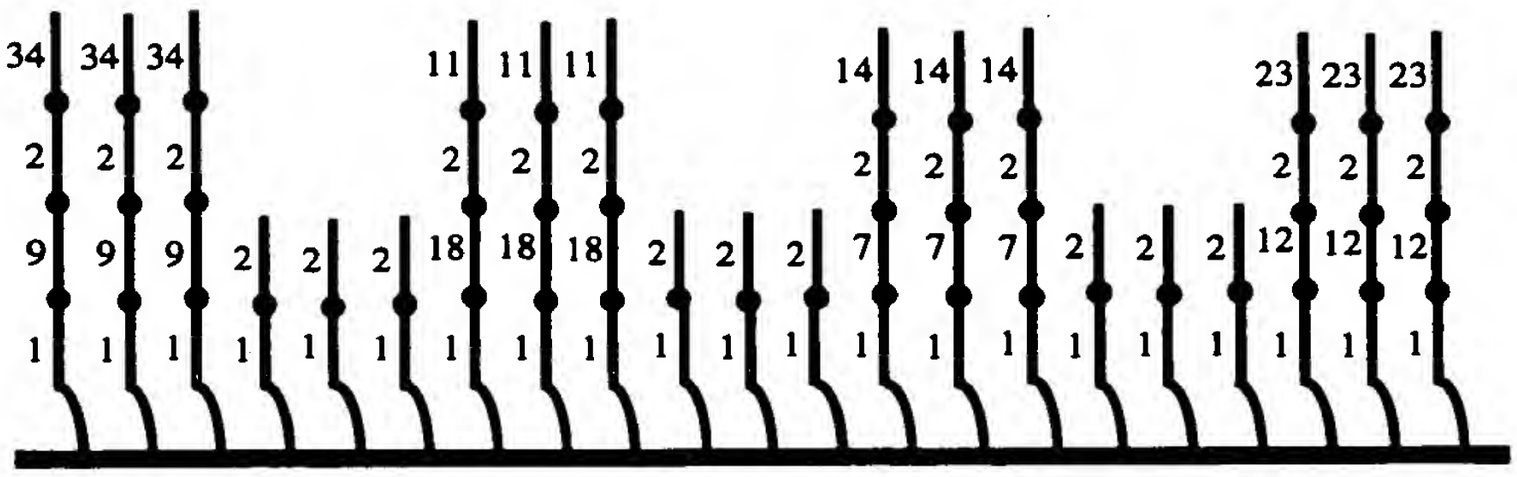
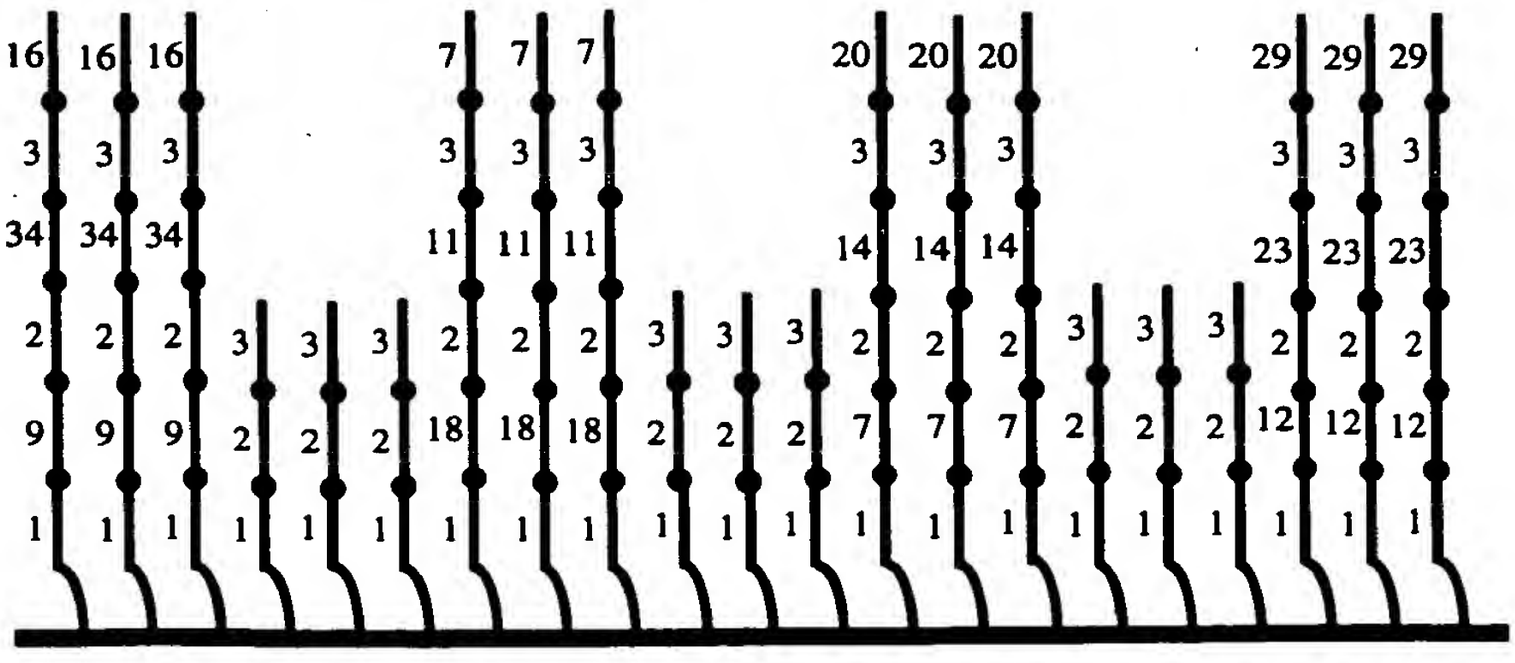


FIG. 21F



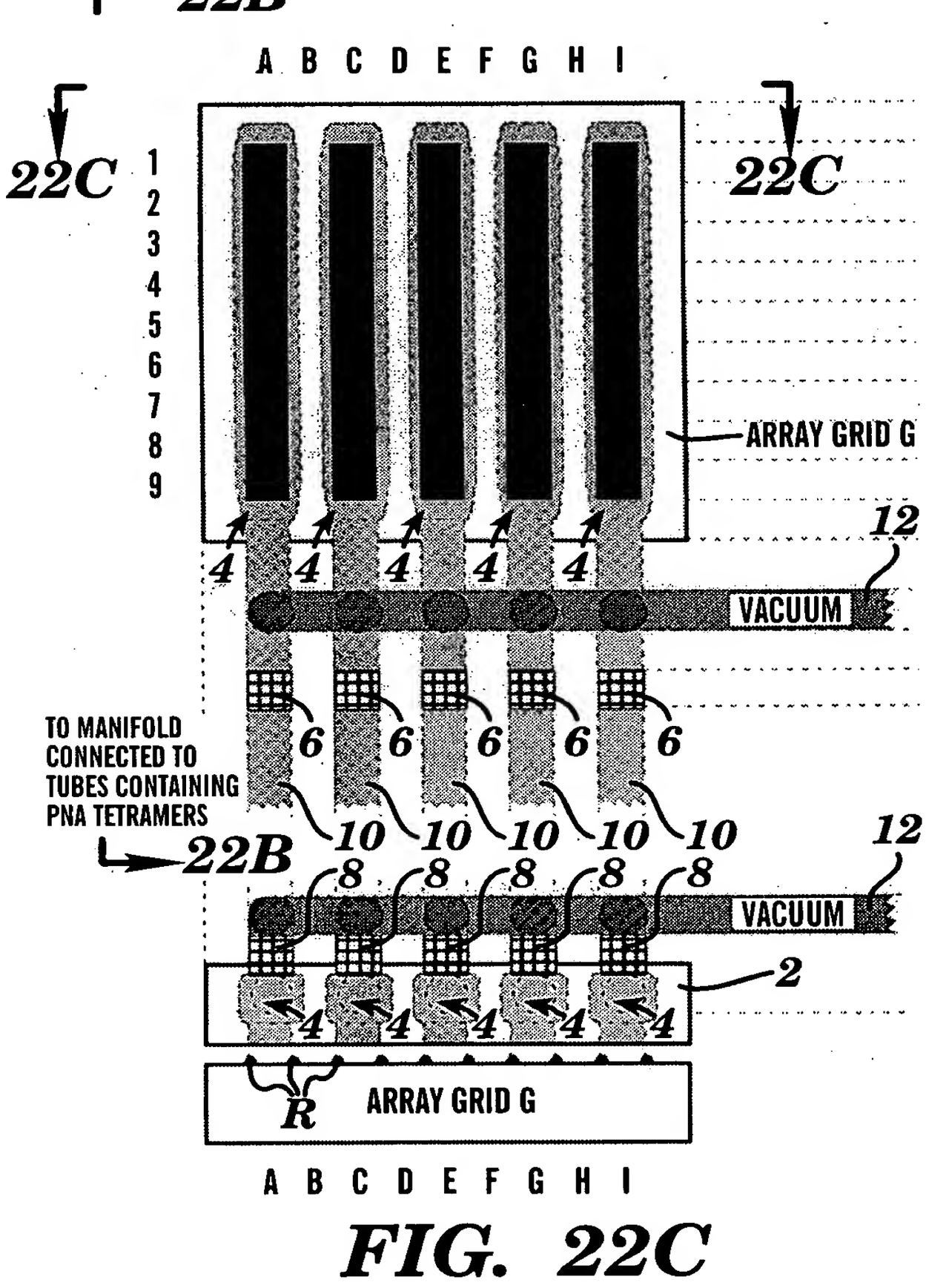


FIG. 22A

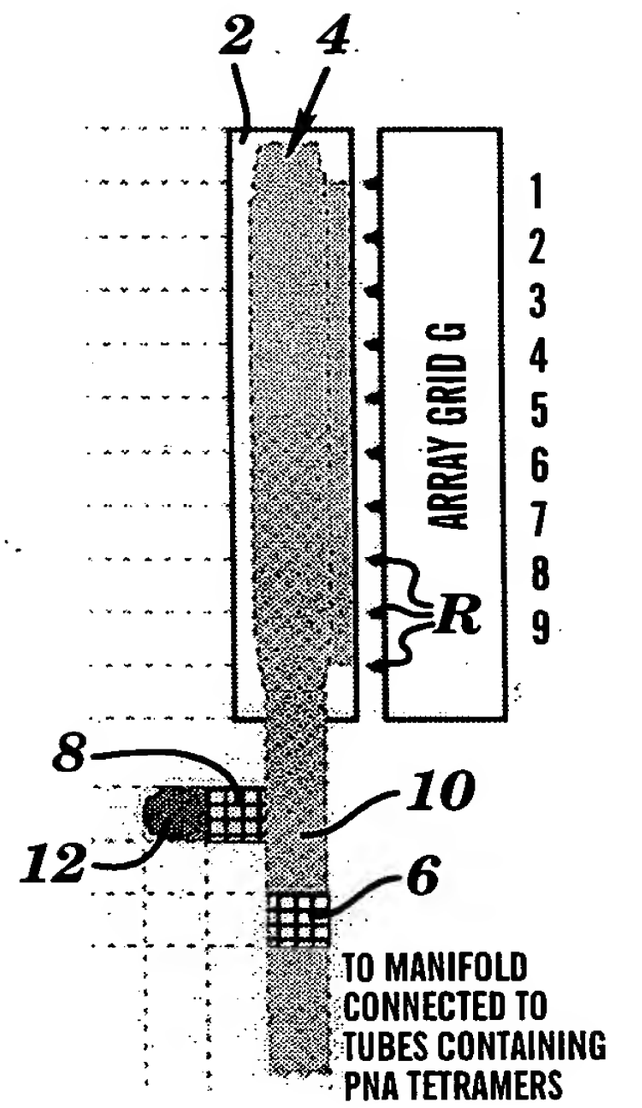


FIG. 22B

FIG. 22C

FIG. 23A

1st Tetramer additions
(columns)

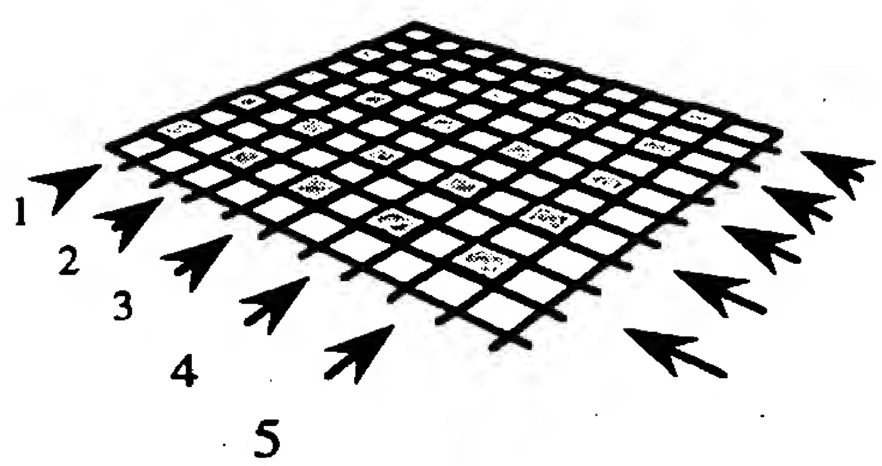
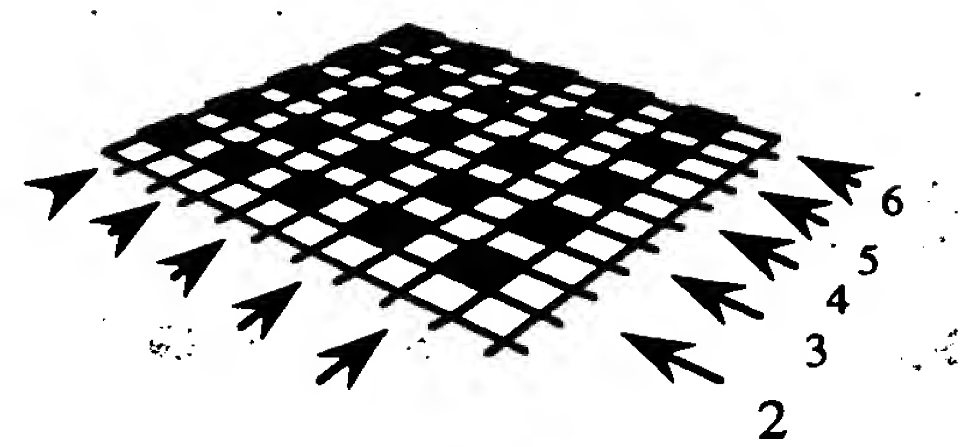


FIG. 23B

2nd Tetramer additions
(rows)



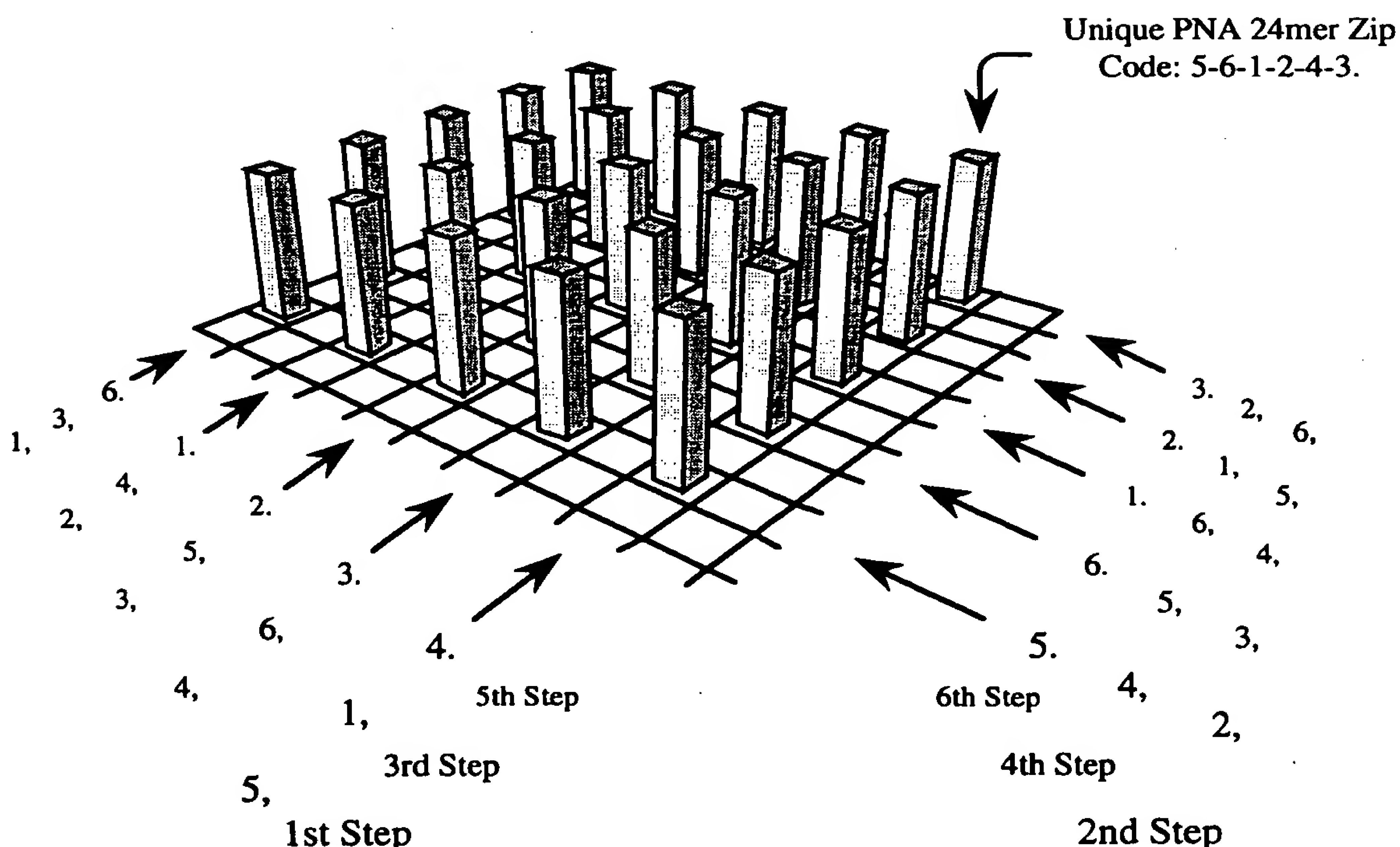


FIG. 23C

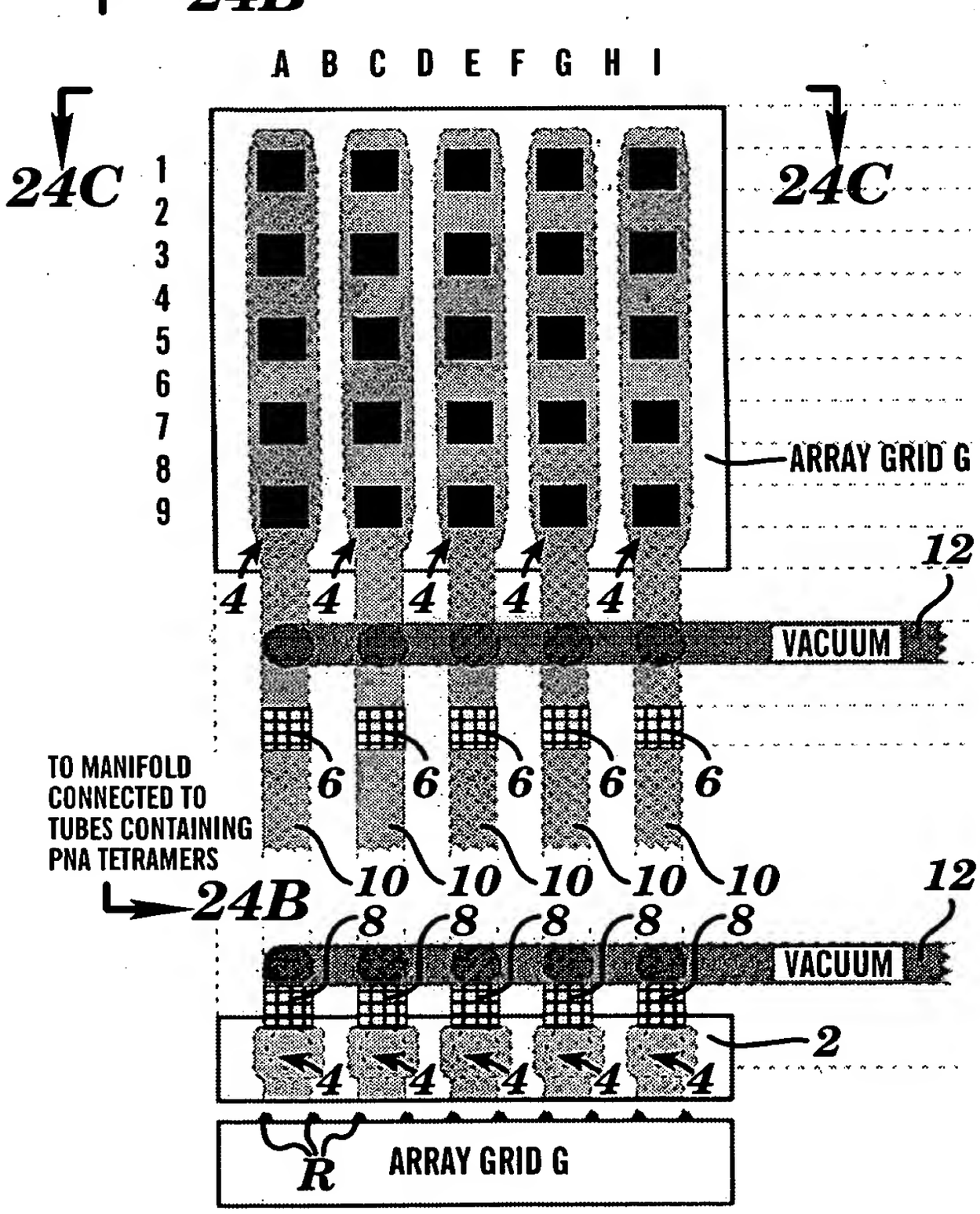


FIG. 24A

FIG. 24C

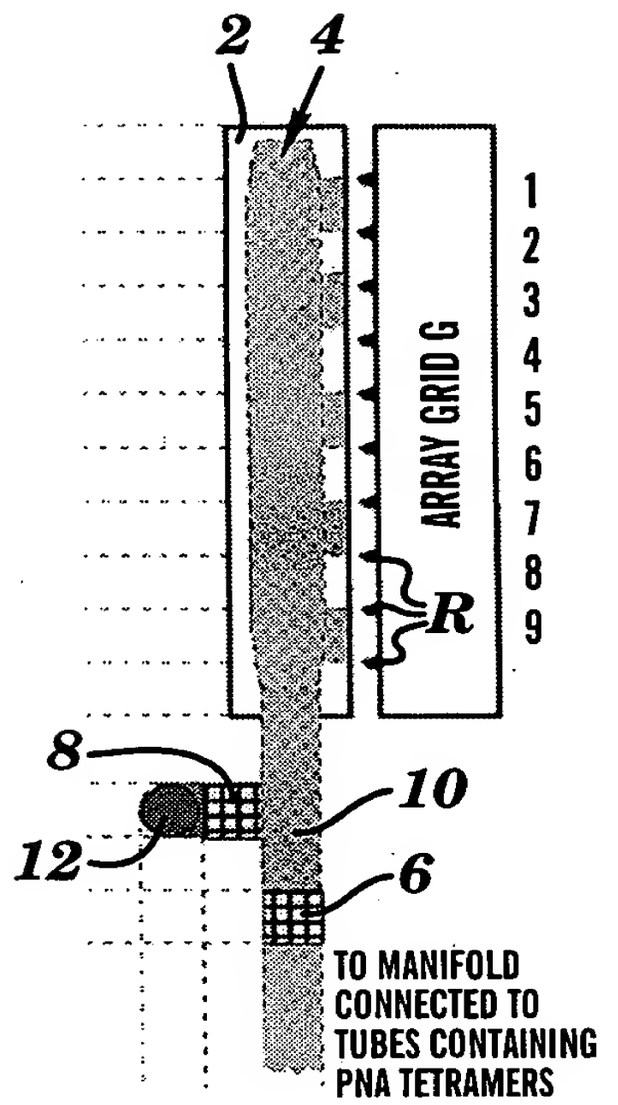


FIG. 24B

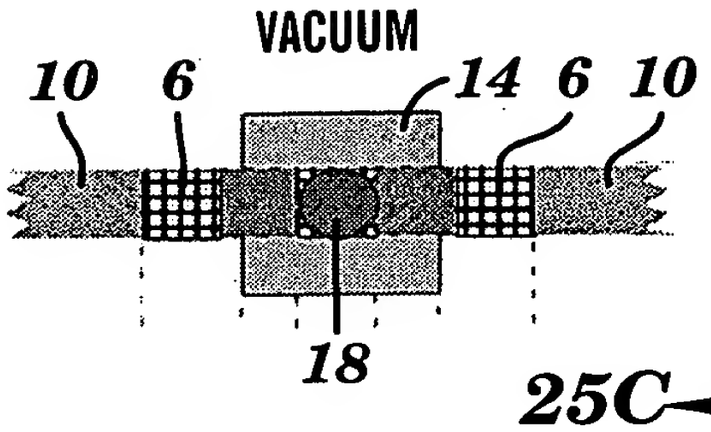


FIG. 25B

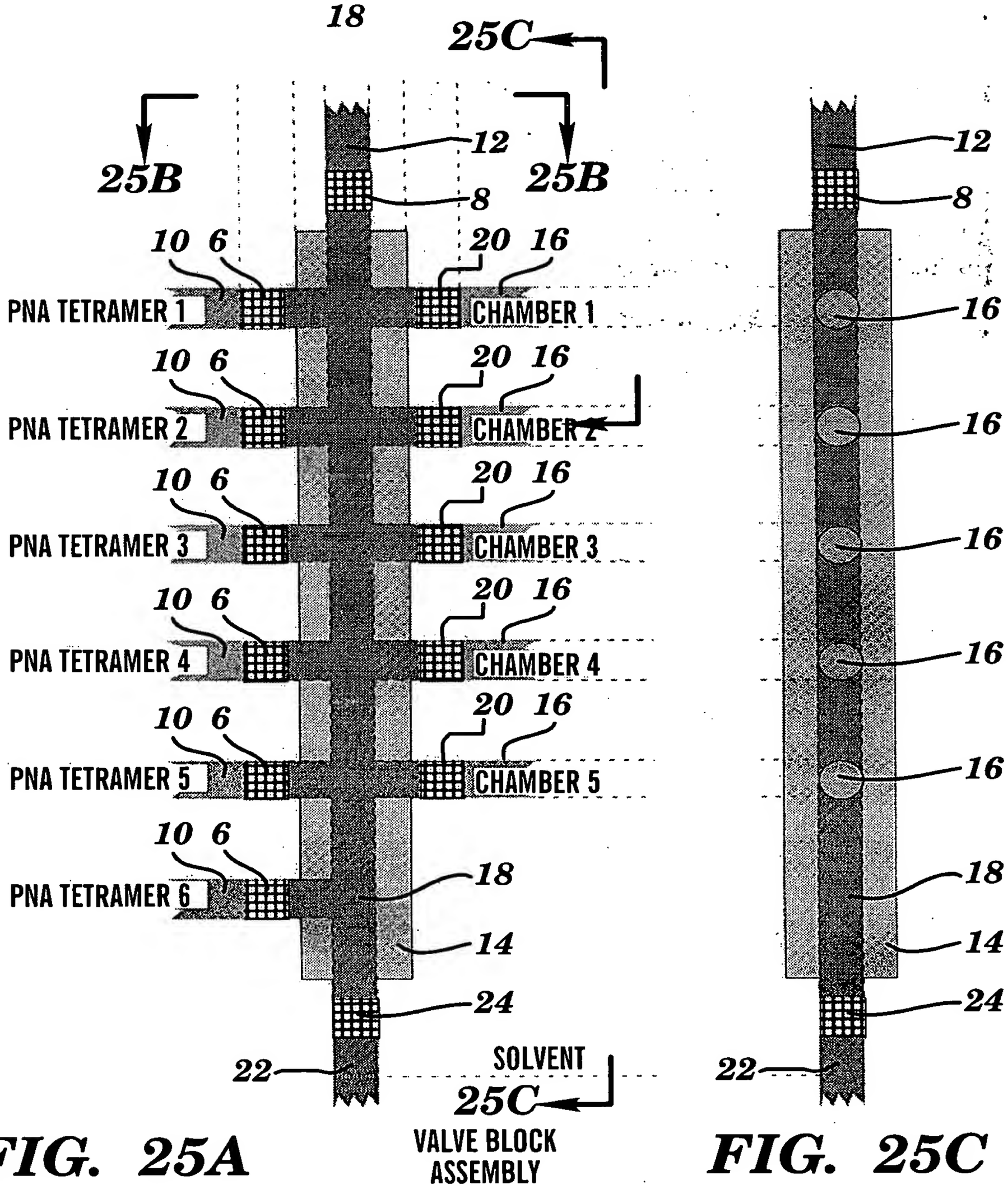


FIG. 25A

FIG. 25C

6 INPUTS AND 5 OUTPUTS

FIG. 26A

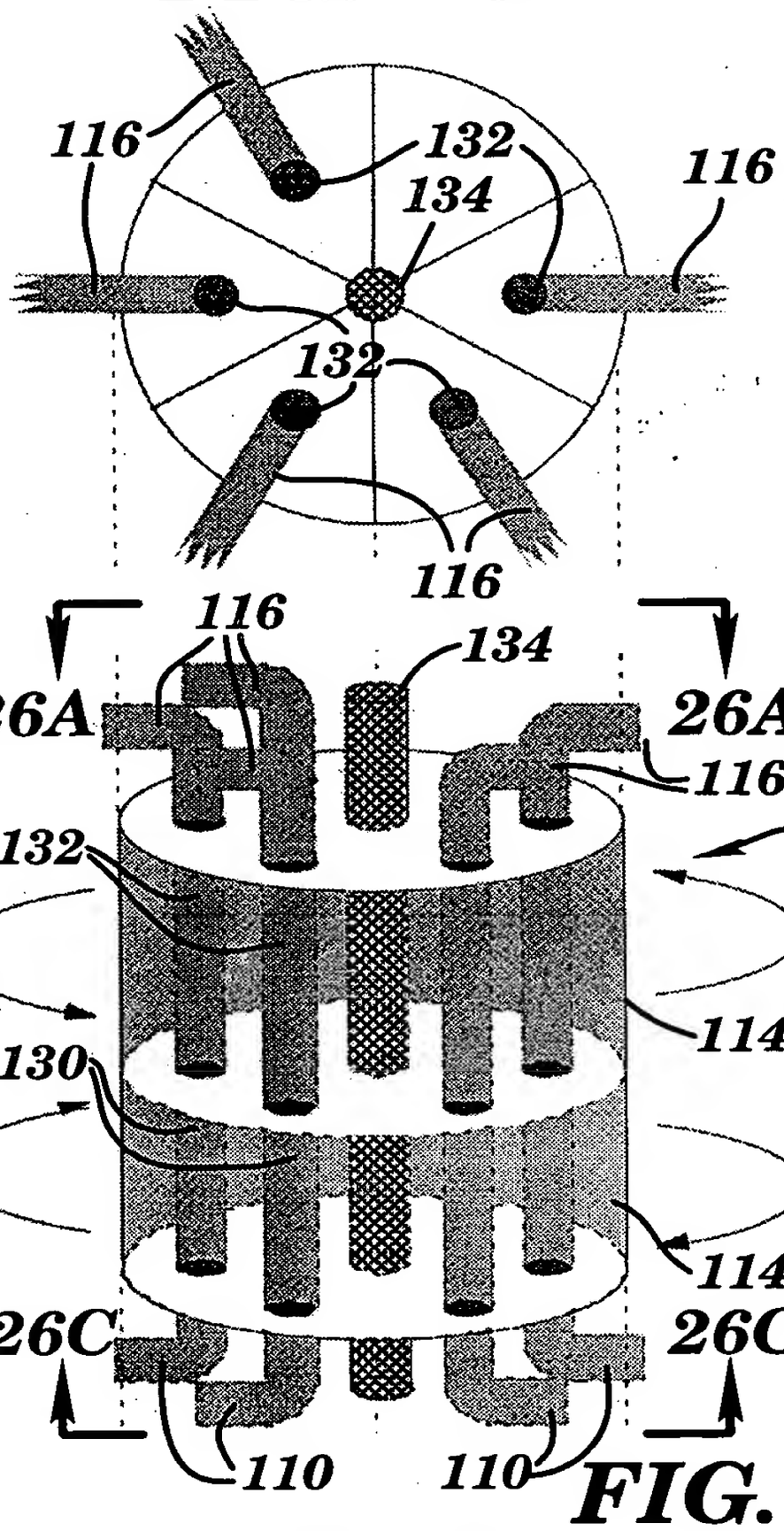


FIG. 26B

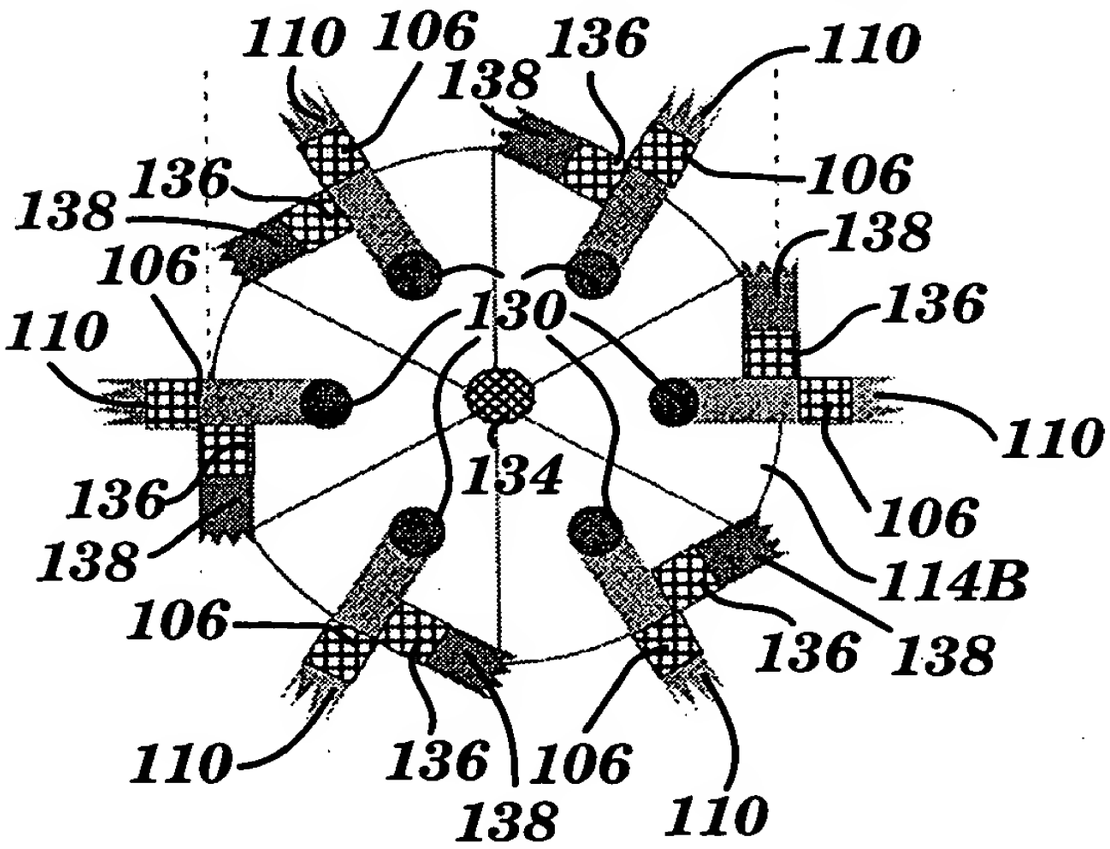


FIG. 26C

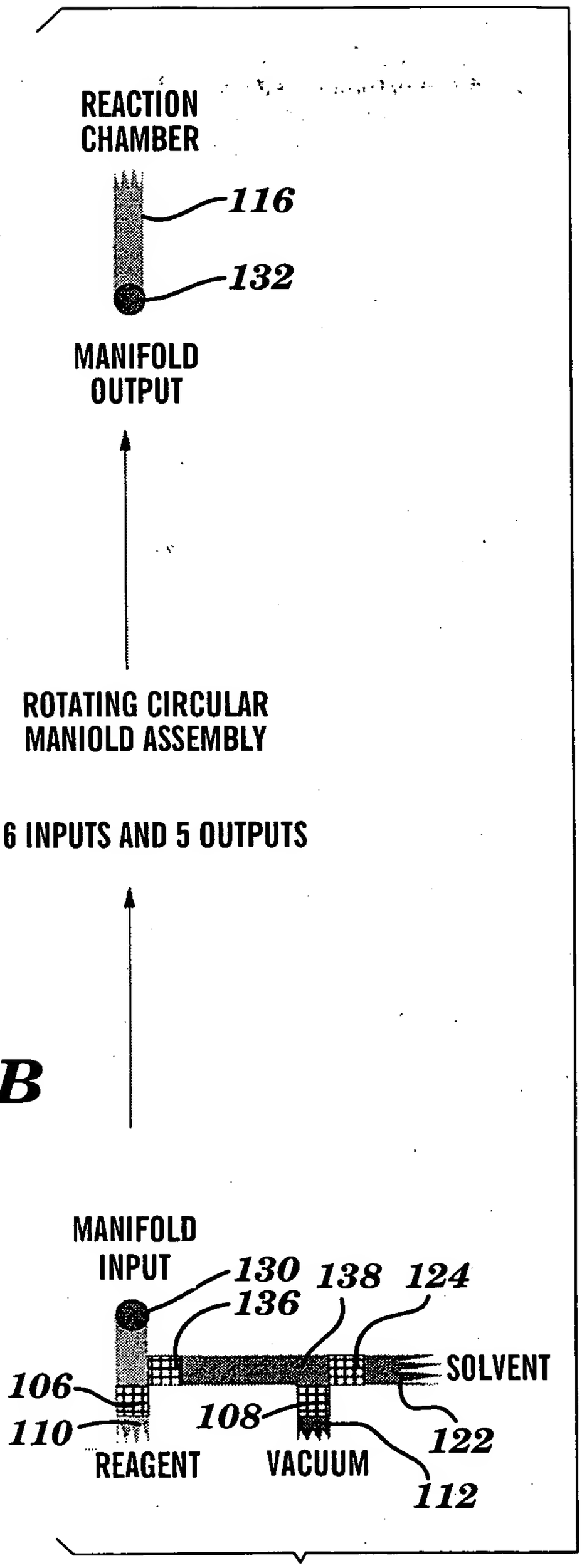


FIG. 26D

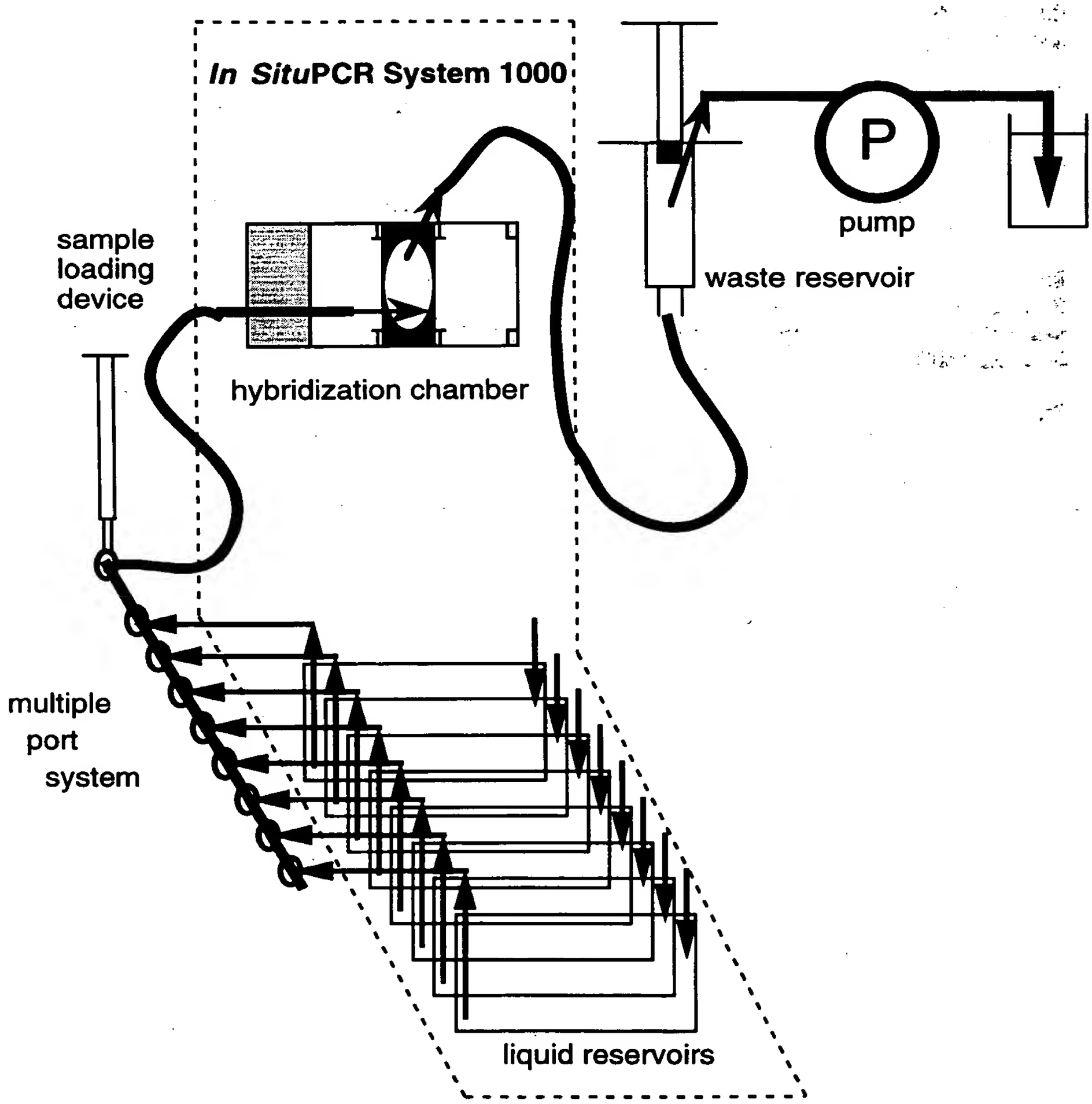

*In Situ*PCR System 1000
sample loading device
hybridization chamber
P
pump
waste reservoir
multiple port system
liquid reservoirs

FIG. 27

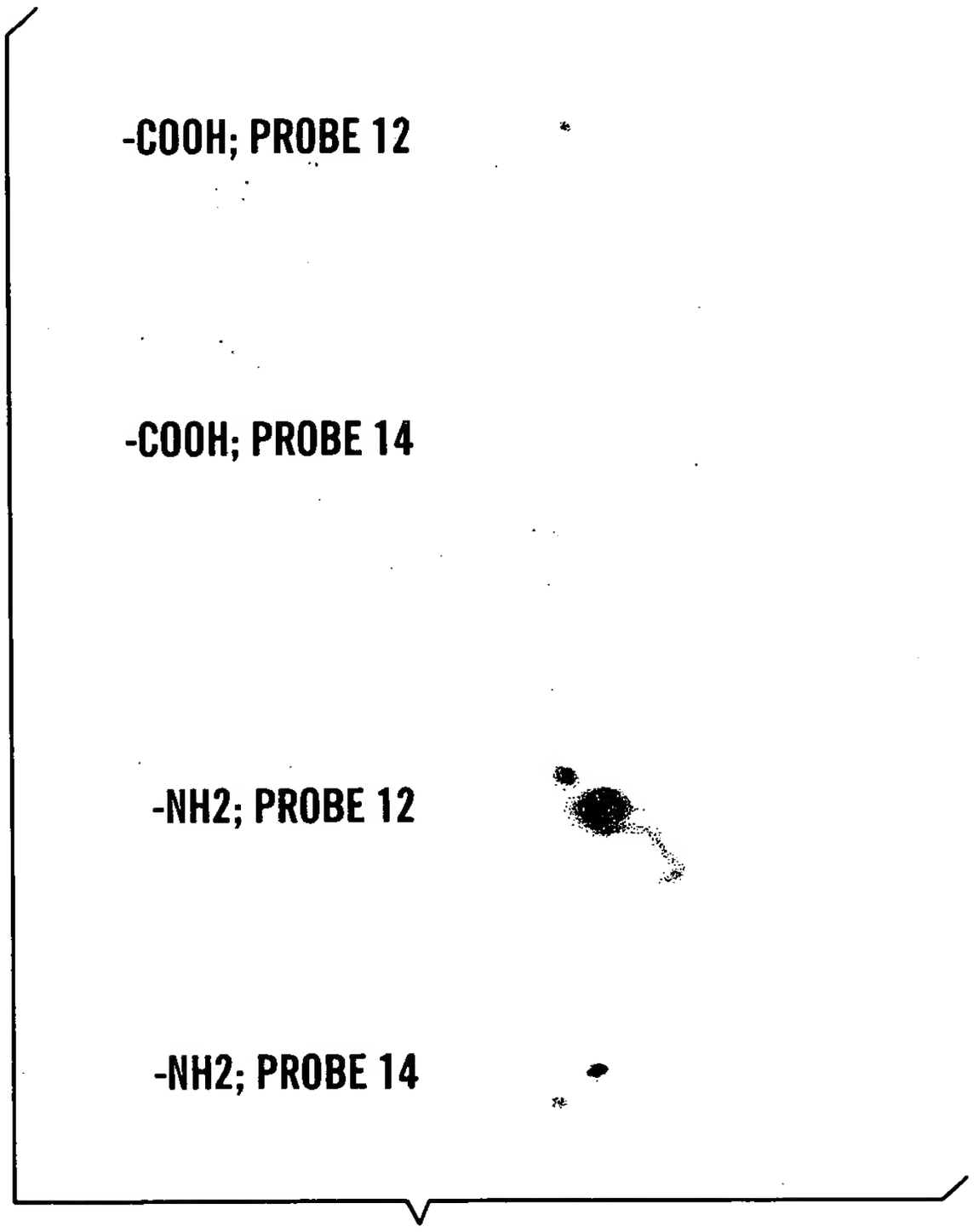


FIG. 28

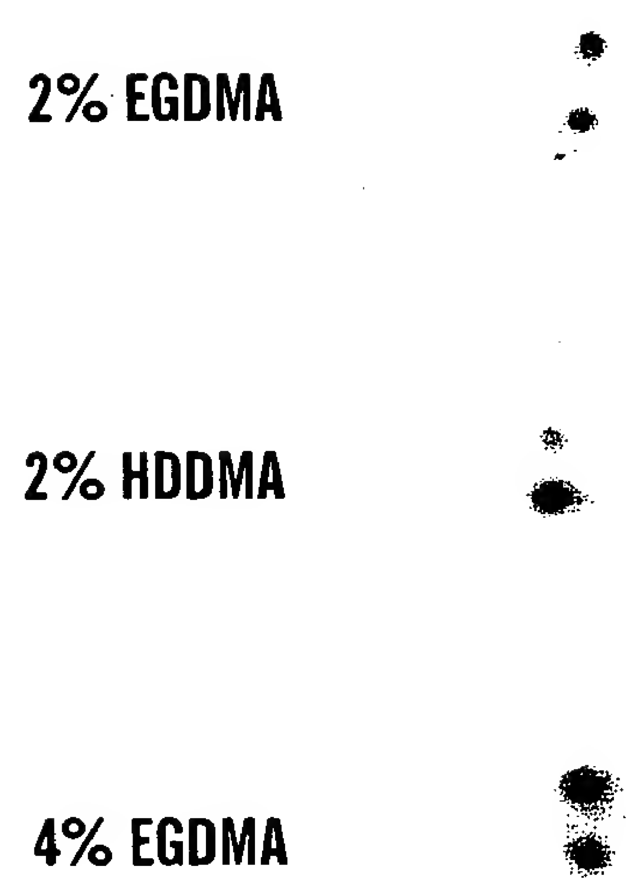


FIG. 29

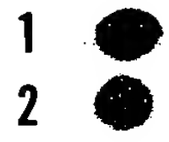


FIG. 30

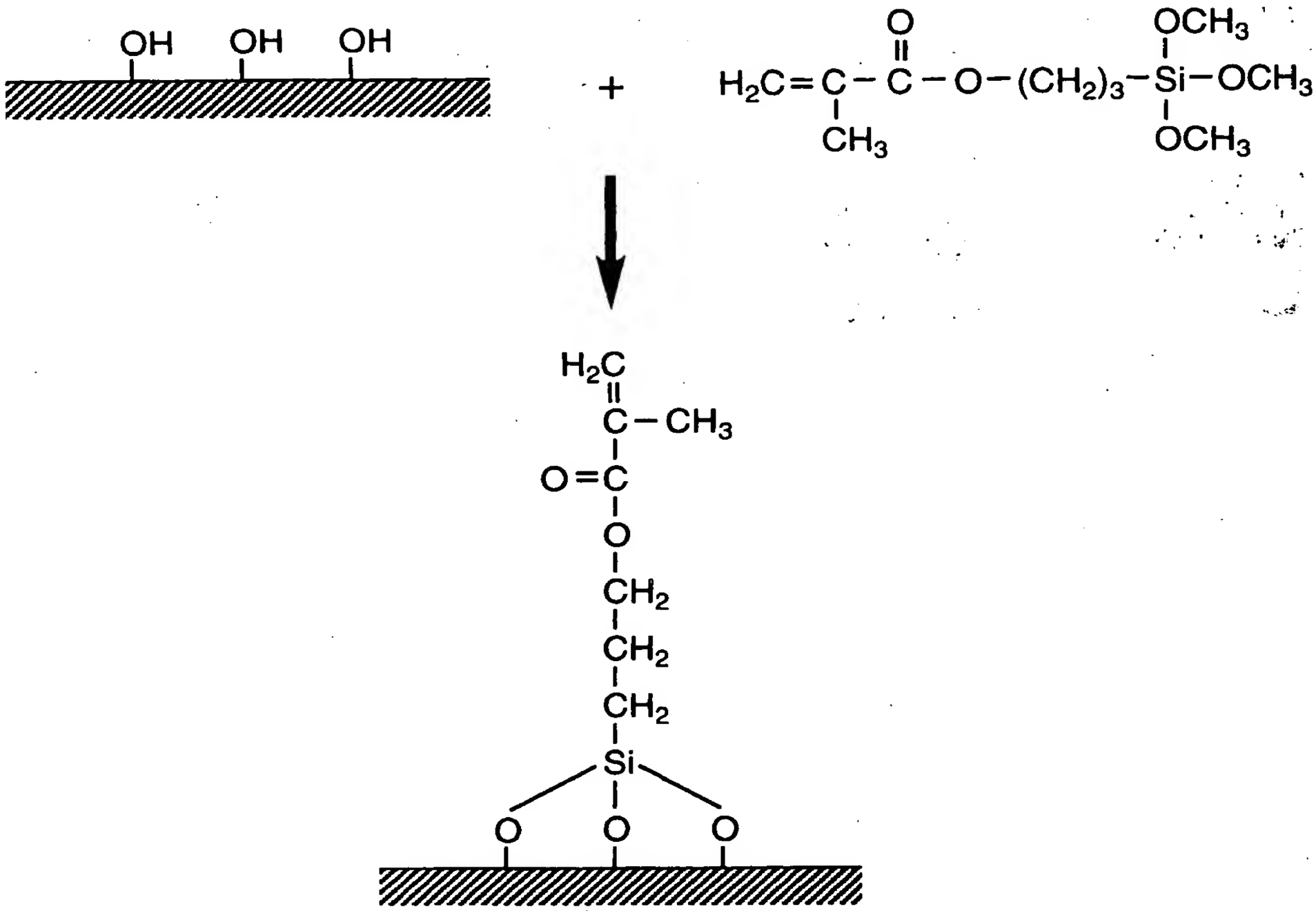

OH OH OH
+
O
OCH3
H2C=C-C-O-(CH2)3-Si-OCH3
CH3
OCH3
H2C
C-CH3
O=C
O
CH2
CH2
CH2
Si
O O O

FIG. 31

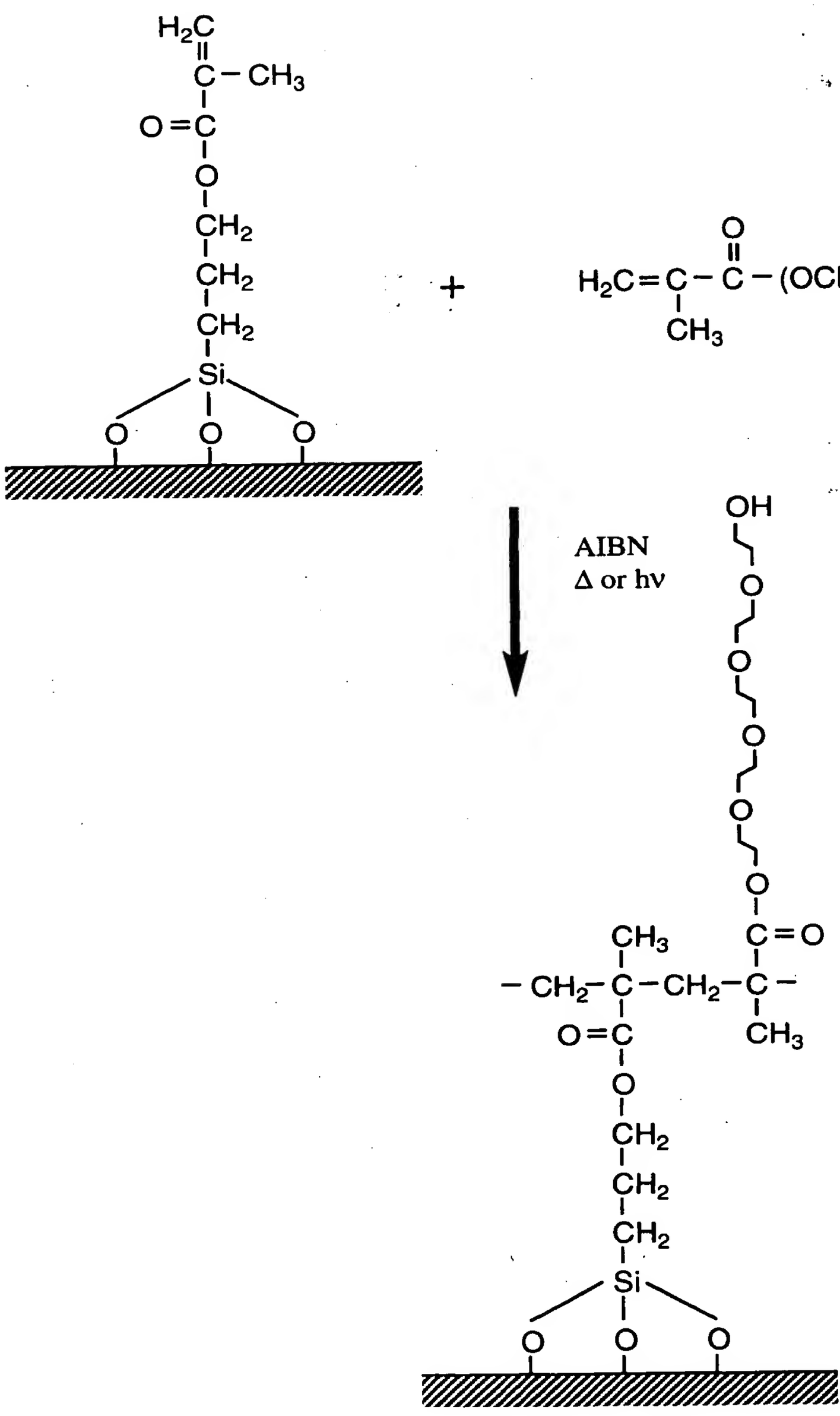

H2C
C – CH3
O=C
O
CH2
CH2
CH2
Si
O O O
+
O
H2C=C – C – (OCH2CH2)n – OH
CH3
n~ 5
AIBN
Δ or hν
OH
O
O
O
O
O
C=O
CH3
– CH2 – C – CH2 – C –
O=C
CH3
O
CH2
CH2
CH2
Si
O O O

FIG. 32

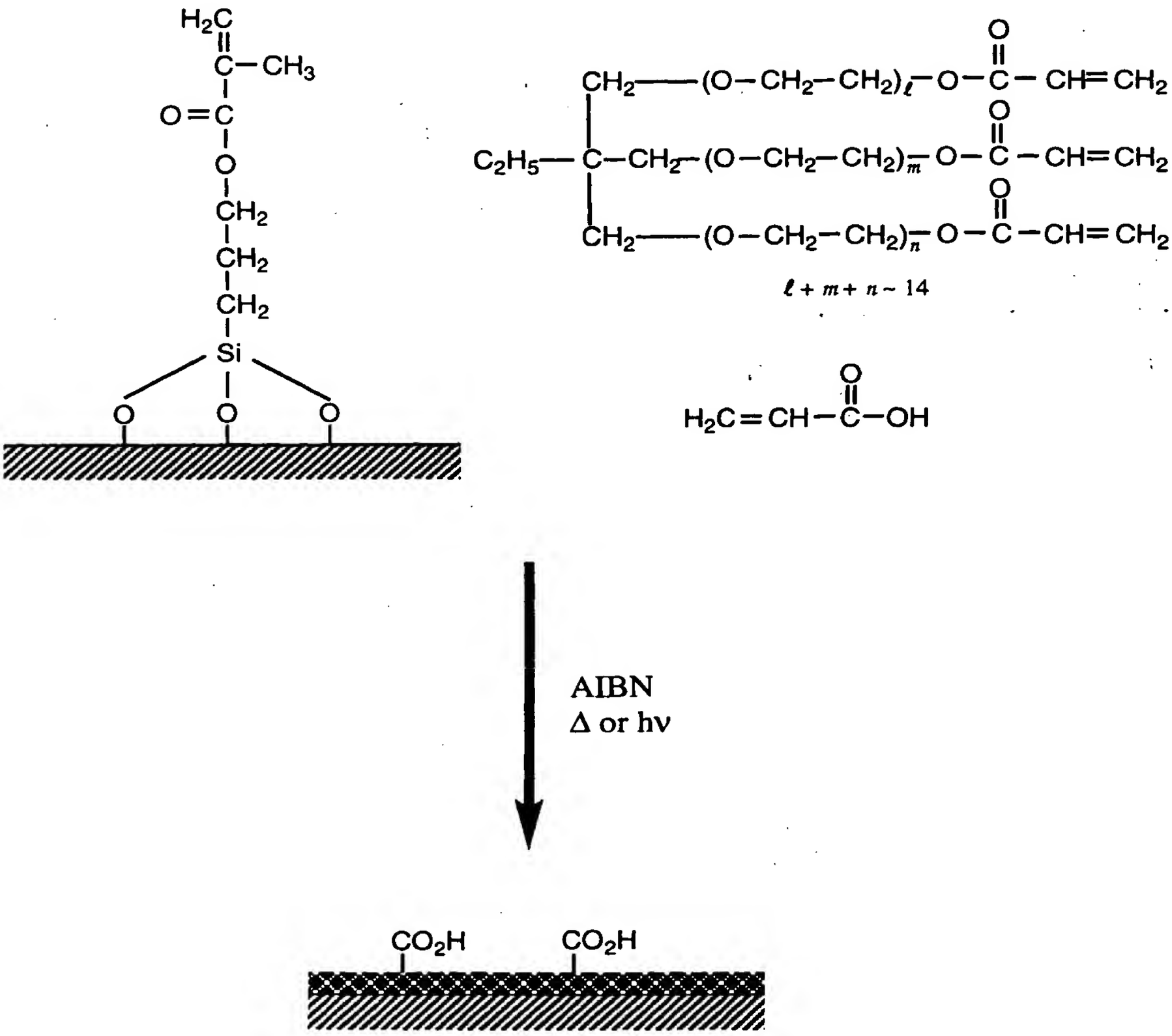

H2C
C—CH3
O=C
O
CH2
CH2
Si
O
O
O
CH2—(O—CH2—CH2)ℓ—O—C—CH=CH2
C2H5—C—CH2—(O—CH2—CH2)m—O—C—CH=CH2
CH2—(O—CH2—CH2)n—O—C—CH=CH2
ℓ + m + n ~ 14
H2C=CH—C—OH
AIBN
Δ or hν
CO2H
CO2H

FIG. 33

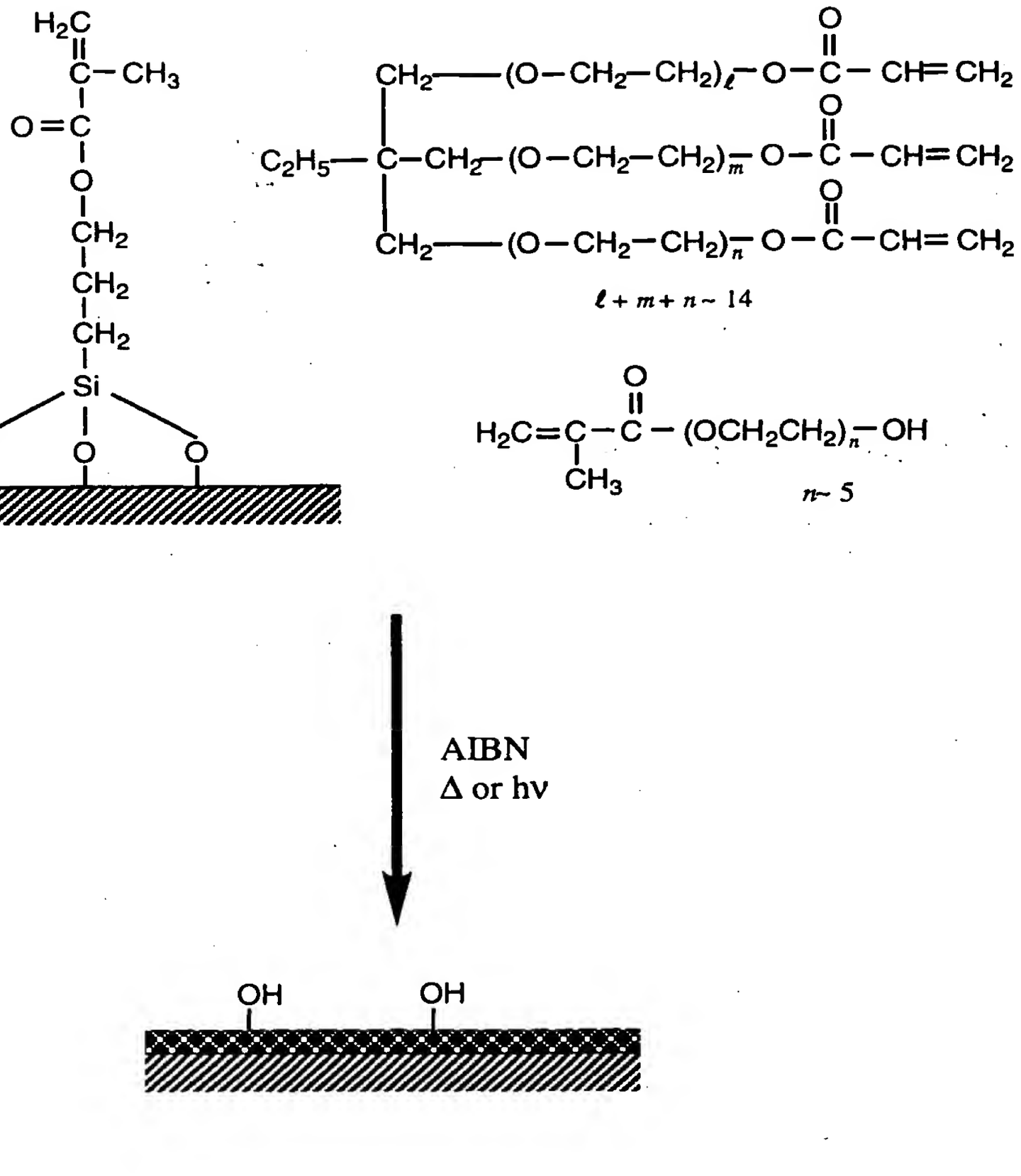

H2C
C–CH3
O=C
O
CH2
CH2
Si
O
O
O
CH2–(O–CH2–CH2)ℓ–O–C–CH=CH2
C2H5–C–CH2–(O–CH2–CH2)m–O–C–CH=CH2
CH2–(O–CH2–CH2)n–O–C–CH=CH2
ℓ + m + n ~ 14
H2C=C–C–(OCH2CH2)n–OH
CH3
n ~ 5
AIBN
Δ or hν
OH
OH

FIG. 34